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EFFECTS OF EXTERNAL TEMPERATURE, MORPHINE, QUININE AND STRYCHNINE ON THYROID ACTIVITY

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All investigators are ready to grant that the thyroid gland is capable of quantitative variations of function and some maintain that under abnormal conditions it may, as well, vary qualitatively. The more these controlling factors become evident, the clearer will be our understanding of the true physiology of the thyroid gland.

Seidell and Fenger (1) demonstrated a seasonal variation in the iodin content of the thyroid glands of cattle, sheep and hogs, the amount of iodin being greatest in the late summer while the minimum was reached in February and March. Since it has been found by Marine and Lenhart (2) and others that the stainable colloid of the thyroid varies directly with the iodin content of the gland and inversely with the degree of hyperplasia, it is probable that this seasonal variation in the iodin content of the gland represents a variation in the activity of the gland itself. Thus in the summer the gland would tend to assume a resting type with a storage of colloid material while in winter a degree of hyperplasia would develop with a corresponding diminution in the iodin content. In producing this seasonal variation in the activity of the gland different factors might play a part: Variations in the composition of the food at the different seasons; difference in the climatic conditions of the various seasons other than temperature differences; and differences in the temperature alone.

Cramer (3) observed that while considerable histological variations are found in the thyroids of rats living under natural but experimentally

uncontrolled conditions, these variations disappear when the animals are housed in a warm room kept at a constant temperature of 20° to 25°C., carefully handled and fed regularly on a suitable diet. He found the gland vesicles then to be somewhat distended with a well-staining colloid and lined with cubical epithelium. By exposing the animals to cold for several days he found that the gland vesicles became collapsed, the lining epithelium became columnar and the colloid lost its affinity for certain stains. This would seem to indicate that changes in the thyroid gland similar to those occurring in its seasonal variations can be induced by artificially regulating the temperature of the environment. It was with the idea of discovering just how extensively variations in this one factor of the environment would serve to produce changes similar to those resulting from seasonal variations that the present work was undertaken.

Also it was desired to know whether, in external temperature variations, we could not have here a means of producing at will either an active or resting type of thyroid. Many attempts have been made to find agencies which would produce such changes and some degree of success has resulted, more so with producing hyperplastic changes than with reducing the activity of the gland. Hunt (4), C. Watson (5), Marine (6), Bensley (7) and Burget (8) all found that hyperplastic changes could be induced by altering the diet of the animals, especially by increasing the protein percentage of the diet. Mansfield and Ernst (9) and Martin, Loevenhart and Bunting (10) obtained such changes as a result of a decreased oxygen supply to the animals. Regarding the resting type of thyroid, Marine (11) obtained distinct changes in that direction almost at will by means of iodin medication, while many workers have produced like results by thyroid feeding. Jackson (12), by subjecting animals to periods of inanition, produced changes in the epithelial cells of the thyroid similar to those usually taken as indicating decreased activity of the gland. Therefore it was thought that it would be of value if it could be found that changes in either direction could be produced at will simply by variations in the temperature of the environment.

METHODS

Young growing rabbits were mainly used in order that there might be some measure of their basal metabolism as evidenced by their rate of growth. However, adult rabbits were also used as well as guinea pigs and cats, in order to show that the findings were not peculiar to young rabbits.

The animals were kept in a room with a fairly constant temperature of 12° to 18°C., and fed regularly on a constant diet for several days or sometimes even weeks before beginning the treatment in order to bring the glands as nearly as possible to a uniform condition in each case. At the end of the pre-experimental period a control specimen of the thyroid of each animal was removed aseptically under ether anaesthesia, fixed, sectioned, stained and kept for comparison with the specimen taken from the same gland after the treatment. For the effects of cold these same animals were placed in a cold room or out-of-doors at a temperature of −5° to + 10°C. and kept there for periods of time varying from three to thirty days. For heat treatment a well-ventilated hot-box with a temperature of 27° to 37°C. was used, with the duration of the treatment varying as above. At the end of the experimental period a portion was removed from the thyroid of each animal, prepared as above and compared with the first specimen taken from the same animal.

Differences in the histological appearance of the secreting cells and the colloid of the gland were considered in this work to represent differences in the activity of the gland at the time the specimens were taken.

Von Orth's fixative was used on the tissues at first but it was found that alcohol-formol fixative (10 per cent formalin in 80 per cent alcohol) gave practically the same results so far as this work was concerned, so the latter fixative was used for most of the tissue specimens.

A record of the daily weights and rectal temperatures of the animals was kept in several series. The diet was kept constant during pre-experimental and the experimental periods.

TABLE 1

ANIMAL	DAYS OF TREATMENT	TEMPERATURE	COLLOID	APPEARANCE OF CELLS	
				Before	After
		°C.			
Rabbit 10	10	30–36	Increased	Cuboidal	Flattened
Rabbit 39	16	31–39	Increased	Cuboidal	Flattened
Rabbit 42	16	31–39	Increased	Cuboidal	Flattened
Rabbit 49	8	31–34	Increased	High cuboidal	Flattened
Rabbit 50	8	31–34	Increased	High cuboidal	Slightly flattened
Cat 9	3	30–35	Increased	Low columnar	Medium to low cuboidal
Cat 14	15	28–35	Increased	Low columnar	Cuboidal
Guinea pig 2	10	27–38	Increased	Cuboidal	Low cuboidal

RESULTS

a. Effects of high external temperatures. The above table of results indicates the changes produced in eight typical examples taken from the total list of thirty three animals subjected to high temperatures. Twenty eight rabbits, two cats and three guinea pigs were treated in this way and in this total there were only four instances in which no

TABLE 2

ANIMAL	DATE	EXTERNAL TEMPERATURE	RECTAL TEMPERATURE	WEIGHT
		°C.	°C.	grams
Rabbit 49.....	October 25	18	38.9	980
	October 26	31–34	39–1	940
	October 27	31–34	39.8	990
	October 28	31–34	40.3	915
	October 29	31–34	39.1	965
	October 30	31–34	40.3	975
	October 31	31–34	39.1	955
	November 1	31–34	41.4	960
Rabbit 21.....	June 2	18	38.9	720
	June 3	12–15	No marked variations	800
	June 4	12–15		810
	June 5	12–15		815
	June 6	12–15		850
	June 7	12–15		850
	June 8	12–15		930
	June 9	12–15		930
	June 10	12–15		925
	June 11	12–15		945
	June 12	12–15		985
	June 13	12–15		1000
Rabbit 9.....	May 6	18	39.0	575
	May 8	15–18	No marked variations	570
	May 11	15–18		615
	May 13	15–18		650
	May 14	15–18		650

changes in either the character of the cells or colloid content of the vesicles were noted. In no case was there either a decrease in the amount of colloid or an increased height of the epithelial cells. Typically, the changes were as follows:

The colloid of the vesicles increased in amount, became more uniform in appearance and took on the stain more readily. Vacuoles were often present in the outer portion of the colloid mass, more espe-

cially in the glands of the animals used during the winter months. In almost every instance these vacuoles disappeared entirely or decreased in size and number during the treatment. The epithelial cells lining the vesicles usually became more flattened, with nuclei and cytoplasm more densely staining, and in no instance were the cells observed to be increased in height.

Accompanying these microscopic changes in the thyroids, there were also other changes in the animals. If the temperature of the hot-box was kept at 30° to 35°C., it was found that growth in these animals was much slower than in the controls, that the fur became dull and unkempt and that the appetite seemed to decrease as the

TABLE 3

ANIMAL	DAYS OF TREATMENT	TEMPERATURE	COLLOID	APPEARANCE OF CELLS	
				Before	After
Rabbit 3.....	17	−10 to + 7°C.	Decreased	Low cuboidal	Medium cuboidal
Rabbit 44....	21	−5 to +10°C.	Decreased	Flattened	High cuboidal
Rabbit 51....	30	−5 to +10°C.	Decreased	Flattened	Cuboidal
Rabbit 53....	10	−5 to +10°C.	Decreased	Cuboidal	Columnar
Rabbit 54....	6	−5 to +10°C.	Decreased	Cuboidal	Low columnar
Guinea pig 6.	27	−10 to +10°C.	Decreased	Low cuboidal	High cuboidal
Guinea pig 7.	27	−10 to +10°C.	Decreased	Low cuboidal	High cuboidal

temperature increased. When the temperature of the box was raised so as to be equal to or slightly above the body temperature of the animals and kept at that level for a few days, a loss in weight and emaciation occurred probably as a direct result of the loss of appetite. Death often followed if this condition was continued for very many days. In such instances as these there also occurred a rise in the rectal temperatures of the animals of 0.5° to 2.0°C., which might also have been a factor in producing the loss in weight and emaciation.

The effects of high external temperatures on the growth rate and on the body temperatures of the animals are shown in table 2.

b. Effects of low external temperatures. Table 3 summarizes the histological changes in the thyroids of seven of the twenty animals

subjected to low temperatures. Sixteen rabbits and four guinea pigs were treated in this way and in every instance there resulted either a decrease in colloid with an increased vacuole formation or a heightening of the epithelial cells, or both together. The results given in the table are typical of those obtained in the whole series of animals subjected to low temperatures.

The changes here are just the reverse of those described as resulting from high external temperatures. The colloid decreased in amount, lost its smooth uniform character and showed an increase in the amount of vacuolation. The epithelial cells of the vesicles were increased in height while the nuclei and cytoplasm were not nearly so densely staining as before. The rate of growth of the animals was much

TABLE 4

Quinine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 25...	4	7	60–120	Increased	Low columnar	Cuboidal
Rabbit 26...	2	3	60–150	Increased	Low cuboidal	Same
Rabbit 31...	4	8	90–240	Increased	Cuboidal	Low cuboidal—flattened
Rabbit 32...	4	8	90–240	Increased	Low cuboidal	Same
Rabbit 35...	4	8	60–150	Increased	Medium cuboidal	Low cuboidal
Rabbit 36...	4	8	60–150	Increased	High cuboidal	Medium cuboidal

greater than that of the animals kept in the hot-box and seemed also to be increased as the temperature of the environment was lowered below normal, as is indicated in table 2. However, more work is being done at present to determine the extent of these effects on growth produced by both high and low temperatures. The fur of the animals became thick and fluffy and their excitability appeared to be very high, apparently indicating a high degree of vitality. The rectal temperature was maintained at normal.

c. Effects of quinine, morphine and strychnine. Since changes in the external temperature seemed to affect metabolism as judged by body weights and conditions and thyroid activity as judged by morphology, the attempt was next made to influence metabolism directly by the action of drugs to determine whether the activity of the thyroid

was correspondingly influenced. The pre-experimental period was observed as before while the external temperature and diet were kept practically constant throughout the experimentation.

Quinine was used for its action on the endogenous metabolism and was administered as quinine bisulphate dissolved in normal salt solution at 37°C., 60 mgm. per cubic centimeter, injected intramuscularly. The results obtained are indicated in the following table.

Thus it is seen that quinine treatment resulted in changes that are practically identical with those observed under high temperatures, so

TABLE 5

Morphine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 27.....	5	10	30–90	Increased	Cuboidal	Low cuboidal
Rabbit 28.....	3	4	30–60	Increased	Cuboidal	Low cuboidal
Rabbit 29.....	4	7	60–150	Increased	High cuboidal	Low cuboidal
Rabbit 30.....	4	7	60–150	No change	High cuboidal	Low cuboidal
Rabbit 33.....	4	8	30–90	No change	High cuboidal	Medium cuboidal
Rabbit 34.....	4	8	30–90	No change	Cuboidal	Same
Rabbit 44.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 45.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 46.....	11	16	30–90	Increased	Cuboidal	Flattened
Rabbit 47.....	11	16	30–90	Increased	Cuboidal	Flattened
Guinea pig 6..	3	5	90–120	Increased	High cuboidal	Low cuboidal
Guinea pig 7..	3	5	90–120	No change	High cuboidal	Same

far as the histological changes indicate. Follicular colloid increased in amount, the epithelial cells decreased in height and both cells and colloid became more densely staining.

Morphine was used as morphine sulphate dissolved in normal salt solution at 37°C., 60 mgm. per cubic centimeter, injected hypodermically. The dosage was so adjusted as to cause the animals to lie quietly with shallow respiration for two to three hours following each injection. The results of the treatment are given in table 5.

Here again the changes are seen to be the same as those observed in the thyroid resulting from high temperatures, namely, a tendency to become a resting type of gland.

Strychnine was tried in a few cases and some changes were observed as illustrated in table 6 below. However, due to the rapid excretion of the strychnine and the rather narrow margin between the stages of hyperexcitability and convulsions, the results were not very satisfactory. The drug was injected subcutaneously in a solution of 0.2 mgm. per cubic centimeter for rabbits and 1 mgm. per cubic centimeter for guinea pigs. The attempt was to so adjust the dosage as to just produce a condition of hyperexcitability and repeat it as often as pos-

TABLE 6

Strychnine

ANIMAL	DAYS OF TREATMENT	NUMBER OF INJECTIONS	DOSAGE	COLLOID	APPEARANCE OF CELLS	
					Before	After
			mgm.			
Rabbit 57.....	5	22	0.3–0.4	No change	Cuboidal	Same
Rabbit 58.....	5	22	0.3–0.4	No change	Flattened	Medium cuboidal
Rabbit 59.....	5	22	0.3–0.4	Decreased, more vacuolated	Cuboidal	Low columnar
Rabbit 60.....	5	22	0.3–0.4	Much more vacuolated	Flattened	Cuboidal
Guinea pig 11.	5	20	1–1.5	More vacuolated	Low cuboidal	Medium cuboidal
Guinea pig 12.	5	20	1–1.5	More vacuolated	Low cuboidal	Medium cuboidal
Guinea pig 13.	10	40	1–1.5	No change	Low cuboidal	Medium cuboidal
Guinea pig 14.	1	1	1.5	More vacuolated	Flattened	Cuboidal

sible without causing convulsions. The injection should have been repeated every one to one and a half hours but other work kept this schedule from being followed as closely as it should have been. For that reason the results are probably less marked than they would have been under more ideal conditions.

It will be seen from the table that the vesicles did not change in size during the treatment except in one instance where there was a distinct decrease in the amount of colloid. However, in all but two of the animals the vacuoles in the outer part of the colloid masses of the

thyroid increased in size and number quite noticeably. In all but one instance there occurred an increase in the height of the epithelial cells lining the vesicles. It would seem that here we have a more active type of gland as a result of the strychnine treatment.

DISCUSSION AND CONCLUSIONS

The morphological changes in the thyroid described as resulting from the different forms of treatment in these experiments, are accepted by most observers as indicating variations in the activity of the gland. Although Bensley holds that the colloid in itself is no indication of the activity of the gland, still changes in the amount and character of the colloid and in the appearance of the secreting cells surely serve to demonstrate changes in the activity of the gland during the experimental period. Thus, under high temperatures we see a storage of colloid material in the vesicles together with such changes in the secreting cells as are usually thought to represent a diminished secretory activity. Therefore it is to be concluded from the results of these experiments that an elevation in the external temperature serves as an agency for reducing the activity of the thyroid glands of animals.

However, the mode of physiological adaptation by which these changes in the gland are brought about is not definitely understood. Accompanying the decreased thyroid activity there is a slowing of the endogenous metabolism of growth and also probably of the metabolism for heat production, since heat elimination is made much more difficult by the high external temperature. The interrelation of these three phases is still a subject for speculation.

Means and Aub (13) and DuBois (14) found that thyroid feeding can markedly increase basal metabolism, as measured by the calorimeter, while thyroid deficiency, as in cretin patients, produces a basal metabolic rate much below normal. Mansfield and Ernst (9) have shown that fever in normal animals (dogs and rabbits) is accompanied by increased protein metabolism and also increased total metabolism, while in thyroidless animals there is no increase in metabolism of either kind, the fever being due only to a decreased heat loss. It seems then that the thyroid is closely concerned with all metabolic processes, those for heat production as well as those for protein metabolism.

In the case of high external temperatures, then, the first effect is probably a diminished heat production or exogenous metabolism, caused perhaps by reflex inhibition of the thermo-genetic mechanism,

including the thyroid. The lessened growth rate observed in the animals might then be due to the decreased thyroid activity. There are probably other factors which aid in producing these results, perhaps the activity of the parathyroid and suprarenal glands. However, this work did not include observations on these structures.

In animals subjected to low temperatures there are found histological changes in the thyroid which indicate an increased activity of the gland. Also there occurs a rapid rate of growth together with a seeming hyper-irritability and general condition of high vitality. Since the rectal temperatures of the animals remained normal in the cold environment, there must have been a great increase in the heat production. Thus the process is probably just the reverse of that resulting from high temperatures. The thermo-genetic mechanism is stimulated by the low temperature of the surroundings and, since the thyroid seems to be included in this mechanism, its activity would thus be increased. This increased thyroid activity then very likely causes more rapid growth metabolism, thus accounting for the rate of growth.

By the action of quinine on the animals it was possible to influence principally the endogenous metabolism and observe the results of this on the thyroid activity. As the results indicate, there occurs a diminished activity of the gland. Since quinine has no known action directly on the thyroid, it seems that the depressed protein metabolism must cause the decreased thyroid activity, probably by bringing about a diminished need for the secretion in the body and allowing its storage as colloid in the gland vesicles.

Morphine produces results in the thyroid similar to those following quinine treatment, although probably by a different action of the drug. This drug also has no known action directly on the thyroid but seems to act on metabolism mainly by reducing nervous sensibility and consequently all metabolic processes dependent on nervous influence, especially muscular activity. The thyroid gland, being closely related to total metabolism, is probably then inhibited by the lessened need for its secretion.

Strychnine, although having no known action on the thyroid directly, seems to cause an increased activity of the gland in these experiments. This is probably due to the greatly increased metabolism in the muscles resulting from the action of the drug on the spinal cord, a larger amount of thyroid secretion being needed for this increased oxidation.

The fact that the vacuoles in the outer part of the colloid masses of the thyroid varied so uniformly in these experiments seems to me to be of some importance. Low temperatures and strychnine treatment,

resulting in an increased thyroid activity, also caused an increase in the number and size of these vacuoles in almost every case. Likewise high temperatures, morphine and quinine caused a diminution in the vacuolation. Whether the vacuoles represent areas from which the colloid has been resorbed to supplement the new secretion of the gland in supplying the body needs or whether it represents the deposit of some new substance by the epithelial cells, are questions yet to be answered. The behavior of the vacuoles under the different conditions in these experiments seems to favor the first theory.

SUMMARY

High external temperatures cause a diminished activity of the thyroid glands of animals, as judged by morphology, together with a slowing of the rate of growth.

Low external temperatures, on the contrary, increase the thyroid activity and also seem to cause a faster rate of growth.

Morphine and quinine appear to decrease the activity of the gland, probably as a result of the lessened metabolism and diminished heat production. Strychnine, on the other hand, causes greater thyroid activity, very likely by increasing metabolism through its action on the spinal cord.

NOTE.—This work was begun in the physiological laboratory of the University of South Dakota, under the direction of Dr. A. L. Tatum. I wish here to express my appreciation of his interest and many helpful suggestions during the course of the work under his direction. I also wish to thank Dr. O. O. Stoland for his interest in the work as it was carried on at the University of Kansas.

BIBLIOGRAPHY

(1) SEIDELL AND FENGER: Journ. Biol. Chem., 1912, xiii, 517.
(2) MARINE AND LENHART: Arch. Int. Med., 1909, iii, 66.
(3) CRAMER: Proc. Physiol. Soc., Journ. Physiol., 1916, xxxviii, 50.
(4) HUNT: Journ. Amer. Med. Assoc., 1911, lvii, 1032.
(5) WATSON: Proc. Physiol. Soc., 1907, 1.
(6) MARINE: Journ. Exper. Med., 1914, xix, 376.
(7) BENSLEY: Amer. Journ. Anat., 1916, xix, 37 and 57.
(8) BURGET: This Journal, 1917, xliv, 492.
(9) MANSFIELD AND ERNST: Arch. gesammt. Physiol., 1915, clxi, 399.
(10) MARTÍN, LOEVENHART, AND BUNTING: Journ. Exper. Med., 1918, xxvii, 399.
(11) MARINE AND LENHART: Arch. Int. Med., 1909, iv, 253.
(12) JACKSON: Amer. Journ. Anat., 1916, xix, 305.
(13) MEANS AND AUB: Journ. Amer. Med. Assoc., 1917, lxix, 33.
(14) DUBOIS: Arch. Int. Med., 1916, xvii, 915.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLVII. Gastric Secretion and Urine Ammonia

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Hawk (1) in his series of studies on water drinking has found an increase in urine ammonia on increasing the amount of water during the meals, which he ascribes to a stimulation of gastric secretion. It had been shown by Walter (2) and others that the urine ammonia can be increased or decreased by the feeding of acids or alkalies. Schittenhelm (3), working upon patients with gastric "hypo-and hyperacidity." came to the conclusion that "an increase in the acidity of the gastric juice causes increased ammonia excretion." A. Loeb (4) and Gammeltoft (5), working with patients and normal persons, report some instances of increased urine ammonia after the meal but a greater number in which there was a decrease in ammonia excretion after the meal. The latter investigator also reports a marked decrease in the urine ammonia upon taking sodium bicarbonate with the meal.

The work presented in this paper is an attempt to throw more definite light upon the relation between gastric secretion and urine ammonia.

GENERAL METHODS

Effort has been made to control all factors such as diet, time since the last meal and the water intake, that might influence the excretion of ammonia. Gastric analyses were made every fifteen minutes. Urine was collected in fifteen to thirty minute intervals by catheter in dogs and by voluntary micturition in man. With training and practice on the part of both man and dog, the accuracy of these methods of urine collection cannot be questioned. Controls were made for one-half to one hour preceding the experiment to determine the continuous gastric secretion and ammonia excretion. The work has been done on five men and has been repeated on female dogs with gastric and duodenal fistulas and exposed urethra. Both the Folin and the Folin-

Nessler (6) methods have been used in the determination of ammonia. The determination was always made on fresh urine. Conclusions are based on from three to ten trials of the same experiment in each individual.

TABLE 1

PROCEDURE 400 cc. H_2O WITH MEAL	GASTRIC JUICE		URINE		PROCEDURE 800 cc. H_2O WITH MEAL	GASTRIC JUICE		URINE	
	Free	Total	Amount	NH_3 N		Free	Total	Amount	NH_3 N
a.m.			*cc.*	*mgm.*	*a.m.*			*mgm.*	*cc.*
6.00	15*	20.0*			6.00	12.5	20.0		
6.30	17.5	25.0	12	4.0	6.30	15.0	25.0	15	2.1
Meal 7.00	17.5	25.0	11	4.2	Meal 7.00	18.0	27.5	12	2.3
7.30	0	5.0	28	5.0	7.30	0	5.0	28	4.2
8.00	0	17.5	48	7.2	8.00	0	12.5	72	8.2
8.30	1.0	25.0	90	8.4	8.30	5.0	22.5	140	11.0
9.00	1.0	32.5	44	8.0	9.00	10.0	40.0	40	8.7
9.30	17.5	47.5	30	6.0	9.30	22.5	80.0	32	4.5
10.00	25.0	82.5	28	4.6	10.00	40.0	100.0	24	5.2
10.30	40.0	100.0	20	4.2	10.30	67.5	115.0	16	4.8
11.00	45.0	105.0	20	4.0	Stomach 11.00	75.0	110.0	10	4.1
					empty at				
11.30	60.0	95.0	13	3.0	11.15 11.30	60.0	75.0	11	4.1
Stomach 12.00	45.0	55.0	12	2.5	12.00	37.5	45.0	9	4.3
12.30	30.0	40.0	13	2.7	12.30	27.5	35.0	8	4.0

* The free and total gastric acidity is expressed in clinical units, which is the number of cubic centimeters of N /10 Na OH required to neutralize 100 cc. of gastric contents.

EXPERIMENTAL

Gastric stimulation with absorption in the intestine and urine ammonia

Digestion and absorption of a standard meal with "moderate" and "copious" amounts of water with the meal. The subject, with normal gastric history, was on a diet with a standard meal consisting of 125 grams of graham crackers, 50 grams of peanut-butter, 300 cc. of milk and 400 cc. of water. When 400 cc. of water were ingested with the meal the amount was called "moderate," while the amount was called "copious" when 800 cc. were drunk. Five cubic centimeters of the stomach contents were taken out for each determination of acidity.

The results as recorded in table 1 are typical, in the case of this

individual, for a diet[1] that lasted nine days. The increase in ammonia after the meal along with the diuresis is marked. When the amount of water with the meal was increased to 800 cc., the ammonia excretion was increased. During the period of increased water drinking, the total daily amount of ammonia was increased by 40 mgm., which corroborates the findings of Wills and Hawk (1) and is shown in table 2.

Not only does table 2 show an increase in urine ammonia upon increasing the water with the meal but it also shows that a greater increase takes place when the water ingested with the meal is increased, although the total daily amount of water intake is the same. Referring to table 1, it is seen that the increased excretion of ammonia occurs during the period of the absorption of the water, indicated by the increase in urine amount. Although there is a greater gastric stimula-

TABLE 2

Daily ammonia excretion as influenced by water drinking with the meals

CUBIC CENTIMETERS OF H_2O WITH MEAL	PERIOD II (3 DAYS) MODERATE H_2O (1800 CC.) MILLIGRAMS OF NH_3 N	PERIOD III (3 DAYS COPIOUS H_2O (3600 CC.) MILLIGRAMS OF NH_3 N	PERIOD IV (3 DAYS) COPIOUS H_2O (3600 CC.) MILLIGRAMS OF NH_3N
200	240*		
800		294*	
400			281*

* Average daily amount for the three day-periods

tion when 800 cc. of water are drunk than when 400 cc. are drunk, likewise when 200 cc. are drunk, it might be suggested, since the urine ammonia was not very decidedly increased, that the increase is due to the absorption of the excessive water or to the diuresis. (Observations to answer this suggestion have been made and will be presented later in the paper).

Digestion and absorption of a standard meal. The three subjects in this experiment were not on a standard diet but did ingest the standard meal mentioned above. The results in tables 3 and 4 are typical for a series of six tests performed upon each individual. Table 5 shows typical results for three tests performed on two dogs on a diet of one

[1] The diet was conducted as follows: A preliminary period of two days without control of amount of water; a period of three days of moderate water (1800 cc.) taking 200 cc. with the meal; a third period of three days of copious water (3600 cc.) taking 800 cc. with the meal; a fourth period of three days of copious water (3600 cc.) taking 400 cc. with the meal. See table 2.

TABLE 3

PROCEDURE	SUBJECT N				SUBJECT I			
	Test I		Test IV		Test II		Test VI	
	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour	Urine amount	NH_3 N per hour
p. m.		*mgm.*		*mgm.*		*mgm.*		*mgm.*
1–4	97	15.8	25	32.0	30	12.1	36	14.3
Meal 5.00	32	16.8	26	29.4	49	18.2	47	19.1
6.00	50	18.2	33	30.8	98	23.8	75	21.6
7.00	73	23.4	37	28.6	35	23.8	53	22.4
8.00	35	28.0	36	29.4	28	16.0	32	18.7
9.00	29	23.4	31	29.4	20	14.1	18	14.3
10.00	24	22.5	28	28.5	15	12.4	14	11.5

TABLE 4

PROCEDURE	SUBJECT H				
	Urine amount	NH_3 N per hour	Time	Urine amount	NH_3 N per hour
a. m.		*mgm.*	*p.m.*		*mgm.*
11.00–12.00	54	8.4	4.00–5.00	50	19.6
Meal 12.30	23	4.3	Meal 6.00	37	14.1
1.00	19	4.3	7.00	50	28.0
1.30	70	9.6	8.00	77	32.5
2.00	260	11.2	9.00	38	28.0
2.30	52	6.3	10.00	26	21.0
3.00	34	9.8	11.00	18	18.2
4.00	49	12.6			
5.00	42	8.9			

TABLE 5

PROCEDURE	DOG A		PROCEDURE	DOG B	
	Urine amount	NH_3 N per hour		Urine amount	NH_3 N per hour
		mgm.			*mgm.*
11.30–2.45	51	42	9.10– 9.40	3	4.4
3.15	5	5.4	10.10	3	4.9
Meal 3.45	4	6.1	Meal 10.40	4	5.8
4.15	7	9.5	11.10	5	10.5
4.45	13	19.6	11.40	14	16.6
5.15	22	26.6	12.10	19	17.15
5.45	18	16.8	1.10	42	36.6
6.15	11	15.4	2.10	40	37.0
			3.10	30	30.0
			4.10	15	18.0

meal a day of 200 grams of fresh lean meat ground and mixed with 250 cc. of H_2O.

Subject N, as table 3 shows, gave varying results. This individual, although normal and in excellent health, never gave with an Ewald meal (fractional method) a higher acidity than 0.16 per cent; and with water there was practically no stimulation of the gastric glands. This is offered as an explanation why increase in ammonia output did not occur with this person. Subject H, table 4, always showed an increase in urine ammonia after a meal as did subject I. Both of these individuals gave a marked response to water stimulation and the Ewald meal. Marked diuresis generally occurred. Why the dogs

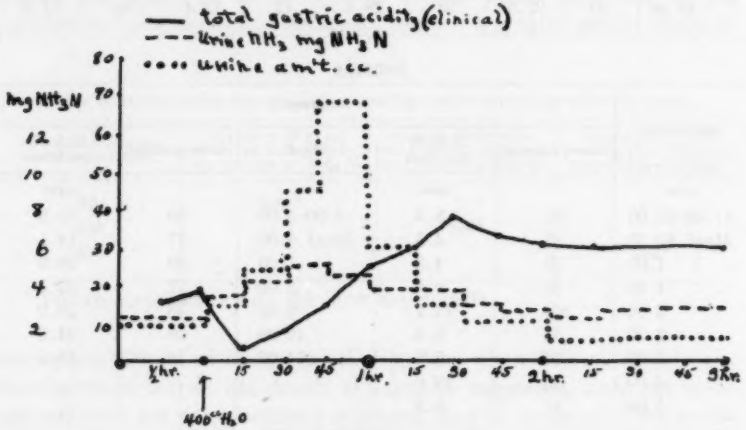

Fig. 1

show such a very marked increase in urine ammonia (table 5) after the meal, has not been determined. But it has been observed as a general observation, without any direct evidence other than that shown in this paper, that the "NH_3 mechanism" in the dogs is more easily influenced by acids and alkalies than in the case of the men worked upon.

Gastric stimulation with water: man and dog. In a series of experiments upon five men, the urine was collected and the continuous gastric secretion was withdrawn previous to the ingestion of the water, after which urine was collected every fifteen or thirty minutes. Four hundred cubic centimeters of tap water were given by mouth and samples were withdrawn every fifteen minutes for analysis. The above figure (fig. 1) shows the composite of ten tests upon one individual.

Although these curves (fig. 1) show an increase in urine ammonia, a diuresis and a slight gastric stimulation, it was recognized that allowing the water to remain in the stomach obscured the true relation between these factors. So it was decided to increase the amount of water used to 700 cc. and to empty the stomach completely at the end of every fifteen minutes, thereby desiring to obtain an increase in gastric response, a closer relation of the curves, a diuresis and at the same time have a measured quantity of water pass into the intestine. The following curve (fig. 2) shows the composite of ten tests upon the same individual used in figure 1.

In the ten tests an average of 500 cc. were withdrawn from the stomach after the end of fifteen minutes. This water was generally slightly

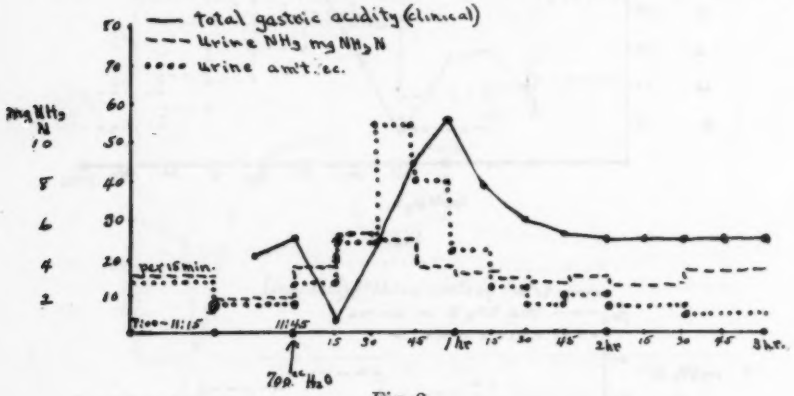

Fig. 2

bile tinged, (Gmelin's test) the latter portion withdrawn being more so. Although this curve shows no great increase in urine ammonia, it is significant because of its constancy and its occurrence when, as shown by controls, the urine ammonia would otherwise be on the decline. This person always showed a decline in urine ammonia during the early morning and forenoon until the mid-day meal was eaten, provided that no water or food was taken in the meantime.

This experiment was repeated upon four other normal men. In two of these the stomach[2] was stimulated by water and the urine ammonia

[2] Work upon this question has shown that all stomachs of apparently normal persons are not stimulated by water. There seems to be some relation between the emptying time and the occurrence of the stimulation, e.g., those stomachs that empty less than 150 cc. in fifteen minutes when 400 cc. are drunk, respond much more than those stomachs that empty more than 150 cc.

was increased. In the other two there was practically no response to water and no increase in NH_3 excretion resulted. Figure 3 shows the

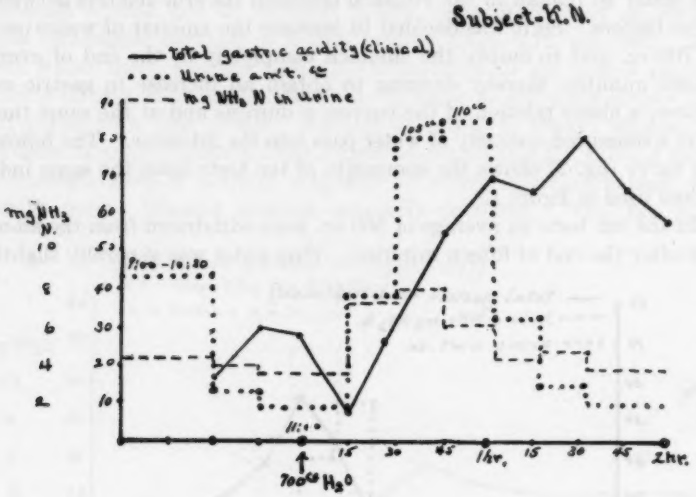

Fig. 3

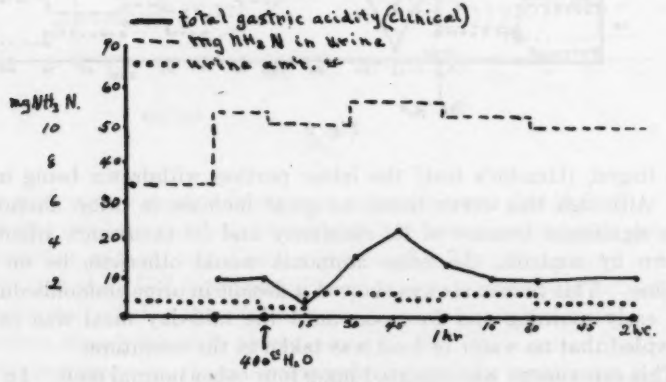

Fig. 4

response in a man whose stomach was stimulated by water, and figure 4 shows the response in a man whose stomach was practically not stimulated by water.

This work was repeated upon four dogs with the urethra exposed for catheterization and similar results were obtained as upon man. Three of the dogs showed increased urine ammonia upon water ingestion, while the fourth showed no change. In the three dogs that did show increased NH_3 excretion it was known that their stomachs responded to stimulation by water as these dogs possessed Pavlov accessory stomachs and had been used in other work. The fourth animal died of distemper before it was found whether the stomach responded to water stimulation. The dogs were trained to take water by stomach tube without being disturbed thereby.

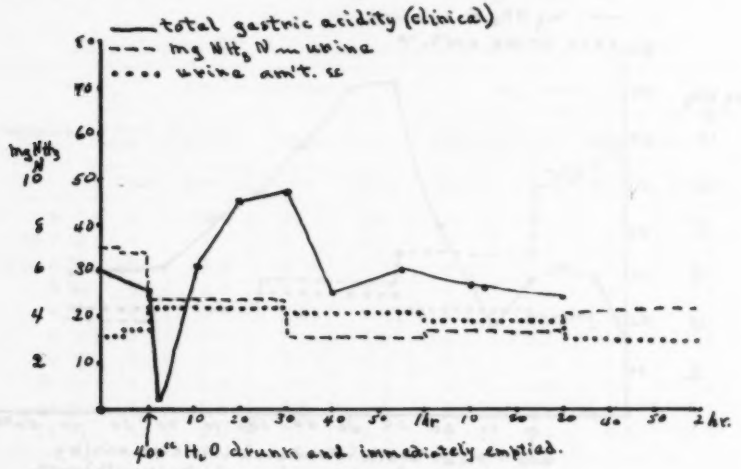

Fig. 5

It is apparent, then, that most of the cases in this series show an increase in urine ammonia upon gastric stimulation followed by absorption in the intestine and that where this increase in urine ammonia does not occur, it has been shown that the acidity of the gastric juice was low or gastric stimulation did not occur.

Gastric stimulation without absorption in the intestine and urine ammonia

Stimulation by water: man. In these experiments 400 cc. of water were drunk and immediately pumped out. The gastric juice resulting from the stimulation was drawn out so as to prevent it from passing into the intestine. The amount of fluid withdrawn was measured and compared with the original amount to make sure that some had passed

into the duodenum. This process of drinking the 400 cc. of water and emptying it from the stomach never required longer than one and a half minutes. Figure 5 is a typical example of the results obtained.

Gastric stimulation by meat broth: man. The same procedure used above in gastric stimulation by water was used here also. Four hundred cubic centimeters of meat broth were drunk and immediately withdrawn from the stomach. The results are shown in figure 6.

Figures 5 and 6 show a gradual fall in urine ammonia upon stimulation of the stomach. This occurred constantly in the one person

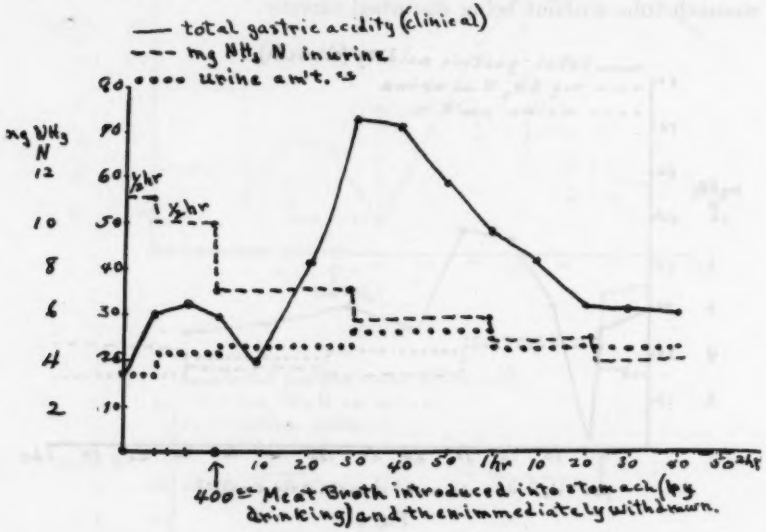

Fig. 6

worked upon. This result is not as significant as it might appear, however, for this individual always showed a decline in urine ammonia during the period of the day in which these experiments were conducted. It does show that there is no increase in urine ammonia upon gastric stimulation.

Gastric stimulation by food: dog. Gastric digestion without absorption. Two dogs[3] with gastrostomy and duodenostomy and perineorrhaphy

[3] If the fistula in the duodenum is made too large, the dogs will not live more than 8 to 11 days. They die of general weakness and debility. The duodenum shows generally pin point ulcers and some enteritis, neither being very extensive

(to expose urethral orifice in order to facilitate catheterization) were fed 100 grams of lean finely ground cooked meat with 100 cc. of water. The food as it left the stomach was conducted from the duodenum, about one and a half inches from the pyloric sphincter, by means of a glass cannula. At times it was found convenient to use an aspirator which was connected to the glass cannula. The emptying time for this meal was generally two and one-half hours (this varied in different dogs) and the amount of chyme collected was generally 75 cc. to 100 cc. more than the amount of semi-fluid ingested. These dogs were dieted during the experimental period.

TABLE 6

Showing urine NH_3 *during gastric digestion without absorption*

PROCEDURE	DOG: PUP, ON DIET				DOG: WHITE, ON DIET			
	Meal: 100 grams meat—100 cc. H_2O		Control, no meal		Meal: 100 grams meat—100 cc. H_2O		Control, no meal	
	Urine	NH_3 N	Urine	NH_3 N	Urine	NH_3 N	Urine	NH_3 N
a. m.	*cc.*	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*
7.00–7.30	9	4	11	6.2	12	6.0	15	8.3
Meal 8.00	8	3.8	10	6.0	10	5.6	13	8.0
8.30	6	3.0	8	5.2	9	5.2	14	8.0
9.00	6	3.1	8.5	6.0	6.5	4.7	12	7.6
9.30	5	2.6	7	5.0	6	4.7	11	7.8
10.00	5	2.6	7	4.8	6	4.2	9	7.0
10.30	4	2.0	7	4.8	5	3.9	9	7.0
11.00	4	2.5	6.5	4.5	4	3.1	8	6.4
11.30	4	2.5	7	4.5	4	3.1	8.5	6.7

or sufficient per se to cause death. Nausea is easily produced by manipulation of the duodenal mucosa. In some instances in dogs that I have observed, when fluid was injected into the distal portion of the gut, a large amount was pushed back through the fistula, unless it was stopped by plugging the fistula with cotton. Violent vomiting was often produced by this latter method and was only relieved by taking the cotton from the fistula and permitting the fluid to flow out. If the fistula is made small (size of a pencil or about 1 cm. in diameter) the dogs will live indefinitely in good health and will not vomit upon injecting fluid, if the latter is injected slowly.

At Dr. A. B. Luckhardt's suggestion this operation has been done in two stages. In the first operation the gastrostomy was made and the first 3 to 4 inches of the duodenum transplanted extraperitoneally. In the second operation a small opening (1 cm. in diameter) was made in the duodenum. Dogs thus operated will live indefinitely. The opening must be dilated daily to prevent closure.

Here again a decrease in the urine ammonia is noticed but a control also shows a gradual decrease. The only conclusion warranted, then, is that there is no increase in urine ammonia during gastric digestion.

Gastric stimulation without absorption followed by the absorption of acid and neutral chyme

Acid chyme: dog. The dogs with the gastric and duodenal fistulas were used in this experiment. The foregoing experiment with them was repeated and the chyme, instead of being thrown away was collected, and when the stomach was empty, it was injected into the distal portion of the intestine for absorption. The chyme titrated from 0.18 per cent to 0.22 per cent total acid and from 0.05 per cent to 0.1 per cent free acid. The free acidity was obtained only toward the latter part of digestion. Tables 7 and 8 show typical results of three trials on each dog.

TABLE 7

Dog P: on diet. Acid chyme. Trial III

TIME	URINE AMOUNT	NH_3 N	REMARKS
a. m.		*mgm.*	
7.00–7.30	2.5	3.2	
8.00	2.2	2.2	Meal: 100 grams ground, cooked meat, 150 cc. H_2O
8.30	2.2	2.2	
9.00	2.2	2.2	
9.30	2.0	2.2	
10.00	1.8	1.7	
10.30	1.8	1.8	Stomach empty; injected the chyme collected into duodenum
11.00	6.5	8.3	
11.30	8.0	12.8	
12.00	10.0	11.0	
12.30	7.0	8.8	
1.00	5.5	7.0	
1.30	4.0	5.1	
2.00	3.0	4.1	

Table 7 shows the slight decline in urine ammonia during digestion referred to before, followed by a marked increase in the urine ammonia and the quantity of the urine output upon the injection of the acid chyme.

Injection of neutral chyme. The foregoing experiment was repeated but instead of injecting the acid chyme, the chyme was made neutral to phenolphthalein by the addition of $NaHCO_3$.

Table 8 shows that the absorption of neutral chyme did not increase the urine ammonia, although a diuresis resulted. On the other hand, there is a diminution of NH_3 after the injection, which is of some significance as it occurred in each of the dogs and in all of the three trials on the same dog.

It is apparent from the results of the injection of acid and neutral chyme into the intestine for absorption that there is an increase in the urine ammonia upon the absorption of the acid gastric contents and no increase in NH_3 upon the absorption of neutral gastric contents.

TABLE 8

Dog P: on diet. Neutral chyme. Trial II

TIME	URINE, AMOUNT	NH_3 N	REMARKS
a. m.		*mgm.*	
7.00–7.30	4.0	4.2	
8.00	4.5	4.0	Meal: 100 grams ground, cooked meat, 150 cc. H_2O
8.30	4.0	4.4	
9.00	3.5	3.8	
9.30	3.5	3.8	
10.00	3.2	3.6	Stomach empty at 10.15. Chyme was neutralized with $NaHCO_3$ and injected into duodenum
10.30	3.0	3.2	
11.00	8.0	3.2	
11.30	12.5	2.0	
12.00	9.2	1.4	
12.30	6.0	1.3	
1.00	4.0	1.3	

Influence of the consistency of the acid chyme upon urine ammonia

As it is well known that water is absorbed in the intestine at a rapid rate and as digestion of food substances by enzymes takes place more rapidly and completely in dilution, thereby facilitating absorption, it was considered of importance to ascertain if the fluid consistency of the chyme had any influence upon urine ammonia.

In one of the duodenal fistula dogs the semi-fluid chyme was collected and the supernatant liquid decanted. This liquid was titrated and its total acidity calculated. The amount of N/2HCl equal to this total acidity was then added to the solid portion of the chyme so that

the amount of the acid in the solid chyme was now equal to the amount of the solid chyme plus the supernatant liquid. The solid chyme was then injected into the duodenum. Table 9 shows typical results of the three trials of this experiment.

As was expected and as is shown by the results in table 9, the consistency or dilution of the acid chyme is a factor in the increase in the urine ammonia during absorption. On diluting the acid chyme the

TABLE 9

Consistency of acid chyme and urine ammonia. Dog P: on diet. Trial III

TIME DECEMBER 24 AND 25	CONTROL: A 325 CC. SEMI-FLUID CHYME		100 CC. OF SEMI-SOLID CHYME B		REMARKS
	Urine, Amount	NH_3 N	Urine, Amount	NH_3 N	
a. m.		*mgm.*		*mgm.*	
8.00					Meal: 100 grams ground cooked meat, 15 cc. H_2O
8.30	8.0	5.3	8.5	3.7	
9.00	7.0	4.7	7.0	3.5	
9.30	6.2	4.5	6.5	3.0	
10.00	6.0	4.0	6.3	3.0	Stomach empty: A, 10.00; B, 10.25.
Inj. 10.30	5.8	3.5	5.0	2.5	Injected into duodenum
11.00	6.0	6.7	5.5	4.8	A, 325 cc. chyme fluid, total acidity
11.30	13.0	11.2	6.0	6.9	140 cc. N/10HCl*
12.00	9.2	8.5	5.0	8.0	B, 100 cc. semi-solid chyme, total
12.30	7.6	6.3	4.5	5.1	acidity 152 cc. N/10HCl*
1.00	6.1	6.0	4.0	4.5	
1.30	6.0	5.8	4.0	4.5	

* All of this acidity is not of gastric juice origin as the meat fed was acid.

urine ammonia was increased as compared with the absorption of more solid chyme, although the total acid content was kept the same. In the latter no free acid was present at all. By way of explanation, dilution facilitates digestion and absorption; and free acid is present. As a result acid is thrown into the blood at a more rapid rate and more ammonia is required to neutralize the acid in order that the H-ion equilibrium be maintained without drawing upon the plasma alkaline reserve.

Absorption of water, acid and alkali from the intestine

In these experiments the subject would swallow at night before retiring two tubes, one of flexible rubber of such a length that it would not be passed into the duodenum, and the other a tube of the Einhorn

TABLE 10

Absorption of water from the intestine: man. Trial II

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.30–7.00	25.0	25.0	32.5	20	6.0	
7.15	10.0	32.5	40.0			
7.30	9.0	27.5	37.5	15	5.1	
7.45	7.5	27.5	37.5			
Inj. 8.00	8.0	25.0	35.0	14	5.0	Injected 200 cc. H_2O into duodenum via duodenal tube
8.15	7.0	22.5	30.0			
8.30	9.0	22.5	32.5	20	4.8	
8.45	6.0	20.0	30.0			
9.00	7.0	20.0	32.5	25	4.6	
9.30	10.0	22.5	30.0	20	4.0	
10.00	12.0	17.5	25.0	18	4.0	
10.30	9.0	20.0	27.5	16	3.5	

TABLE 10A

Absorption of water from the intestine: dog. 150 cc. H_2O

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
2.00			2.00			
2.30	8	5.6	2.30	5.0	2.8	
Inj. 3.00	7	5.0	Inj. 3.00	4.5	3.2	Injection made via the duodenal fistula
3.30	8	6.0	3.30	6.0	3.3	
4.00	12	6.0	4.00	10.0	3.6	
4.30	16	6.4	4.30	16.0	3.6	
5.00	20	5.6	5.00	14.0	4.0	
5.30	14	4.8	5.30	12.0	3.6	
6.00	10	4.5	6.00	7.0	2.5	

type of such a length that 15 inches could pass into the duodenum. The next morning the longer tube would be in the duodenum, which could be accurately determined by blowing air into the tube, or still more accurately by applying suction to the tube in the stomach while injecting fluid into the duodenum. If the fluid injected was drawn out through the stomach tube, the duodenal tube was not in the duodenum

TABLE 11

Absorption of acid from the intestine: man. Trial III

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.30–7.00				13.0	10.8	
7.15	50.0	20.0	35.0			
7.30	10.0	30.0	35.0	12.5	11.0	
7.45	7.0	32.5	40.0			
Inj. 8.00	8.0	32.5	40.0	12.5	9.8	Injected 200 cc. N/20 HCl into
8.15	7.5	30.0	40.0			duodenum*
8.30	7.0	35.0	40.0	19.0	10.8	
8.45	8.0	0	10.0			Bile regurgitation†
9.00	8.0	2.5	12.5	18.5	13.0	
9.30	10.0	35.0	47.5	17.0	11.0	
10.00	11.0	40.0	47.5	16.0	9.8	
10.30	10.0	32.5	37.5	15.0	8.2	
11.00	9.0	30.0	37.5	10.0	7.6	

* There was a regurgitation of bile into the stomach in every trial. A clamp had to be placed on the duodenal tube to prevent bile being forced out upon the clothing.

† This increase in ammonia is not very marked but is significant as it occurred during a time when the urine ammonia was normally on the decline, as stated before for this individual. The largest increase observed during the series of four experiments was 4 mgm. per fifteen-minute period. This table is not published as the gastric record is incomplete.

but in the stomach. Failure of the tube to pass into the duodenum occurred only once during a series of thirty tests. Urine was collected in half hour intervals and the continuous gastric secretion withdrawn every fifteen minutes. Each experiment with acid, alkali and water was repeated three times.

Tables 10, 11, 12 and 13 will show typical results obtained upon one man with the injection of water, acid and alkali.

TABLE 11A

Absorption of acid from the intestine: dog. 150 cc. N/20 HCl

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
1.30	contents		1.30	contents		
2.00	6	4.2	2.00	4.0	5.1	
2.30	5	4.0	2.30	4.0	4.8	
Inj. 3.00	5	4.0	Inj. 3.00	4.0	5.1	Injection made via duodenal fistula
3.30	7	4.8	3.30	8.0	7.6	
4.00	14	9.6	4.00	12.0	8.0	
4.30	20	15.1	4.30	15.0	10.1	
5.00	25	13.2	5.00	19.0	8.5	
5.30	18	8.6	5.30	17.0	9.4	
6.00	12	7.2	6.00	12.5	7.6	
6.30	9	6.1	6.30	7.0	6.2	

TABLE 12

Absorption of alkali from the intestine: man. Trial I

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.00–6.30	36	20.0	37.5	15	8.9	
7.00	14	35.0	45.0	15	8.8	
7.15	7	35.0	42.5			
Inj. 7.30	7	32.5	40.0	20	10.0	Injected 200 cc. 5 per cent $NaHCO_3$ into duodenum
7.45	20	0	1.0			Bile
8.00				19	2.0	Vomited 30 cc. of bile*
8.15						
8.30	20	0	10.0	26	1.8	
8.45	5	0	12.0			
9.00				32	1.6	Stomach dry: no continuous secretion†
9.15						
9.30	5	0	17.5	38	3.0	
10.00	5	7.5	20.0	28	3.0	
10.30	5	15.0	25.0	28	3.0	

* Nausea is very marked and in two instances caused deep abdominal vomiting, expelling tubes and bile. Regurgitation of bile into the stomach always occurred.

† This inhibition resulted only one time.

TABLE 12A

Absorption of alkali from the intestine: dog. 150 cc. 5 per cent $NaHCO_3$

PROCEDURE	DOG A		PROCEDURE	DOG B		REMARKS
	Urine, Amount	NH_3 N		Urine, Amount	NH_3 N	
		mgm.			*mgm.*	
2.00	contents		2.00	contents		
2.30	4	5.4	2.30	6	4.6	
Inj. 3.00	3	5.0	3.00	5	4.6	Injection made via duodenal
3.30	6	4.2	3.30	7	4.0	fistula
4.00	9	2.0	4.00	10	1.8	
4.30	12	0.8	4.30	14	1.2	
5.00	18	1.0	5.00	8	1.0	
5.30	10	0.8	5.30	7	0.8	
6.00	7	1.0	6.00	5	0.8	

TABLE 13

Absorption of water from the large intestine: man. Trial IV

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
9.45–10.15	4.0	12.5	17.5	16.2	5.0	
10.30	4.0	12.5	17.5			
Inj. 10.45	4.0	12.5	20.0	18.5	4.8	Injected per enema of 750 cc.
11.00	20.0	20.0	22.0			warm H_2O into colon*
11.15	8.5	20.0	22.0	16.2	5.3	Bile tinged
11.30	8.0	15.0	20.0			
11.45				13.0	3.0	
12.00	18.0	30.0	32.5			
12.15	5.5	25.0	30.0	9.0	2.1	
12.30	3.4	15.0	22.5			
12.45	4.0	12.5	22.5	9.4	3.0	

* The water was retained in the colon for 15 minutes 500 cc. to 550 cc. of water was in the stool, the other remaining in the intestine.

Table 10 shows that the absorption of water from the intestine causes no change in urine ammonia. Nor, according to table 13, does an increase in urine ammonia take place upon absorption of water from the large intestine, as one might think would happen due to the presence of bacterial decomposition.

The absorption of HCl causes an increase in urine ammonia which corroborates the findings of Walter (2) and others. The decrease in urine ammonia upon the absorption of $NaHCO_3$ (table 12) also accords with the observations of others.

These experiments have been repeated upon dogs with duodenal fistula with identical results (table 10a, 11a, 12a). The results obtained are direct evidence confirming the reports of other investigators that urine ammonia is increased by the absorption of acid, and decreased by the absorption of alkalies and not influenced by the absorption of water.

TABLE 14

Chilling the body by exposure to cold. Trial II

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
6.50–7.20				15	9.1	
7.35	45	20.0	32.5			
7.50	14	32.5	45.0	18	10.0	
8.05	9	32.5	45.0			
8.20	6	25.0	37.5	14	8.4	Began chilling body
8.35	8	22.5	35.0			
8.50	5	25.0	37.5	23	9.2	
9.05	5	22.5	35.0			
9.20	8	17.5	30.0	28	8.8	Stopped chilling body
9.35	5	15.0	25.0			
9.50				15	6.0	
10.20				17	6.2	
10.50				15	6.6	

Diuresis and urine ammonia

Since diuresis accompanied the increase in urine ammonia observed in several of the foregoing experiments, an attempt has been made to see if diuresis in itself might cause an increase in ammonia excretion.

None of the pharmacopeial diuretics proved satisfactory. Upon injection of 20 grains of diuretin, in solution in 20 cc. H_2O into the duodenum, some diuresis resulted with a very marked decrease in urine ammonia. This was due to the sodium salicylate which is said to increase urea synthesis. Injections of water and salt solution intravenously did not prove satisfactory diuretics. It was found that

chilling the body by exposure to cold was the best and least troublesome method to produce diuresis.

It is apparent from table 14, as well as from table 10, that the diuresis per se has no important influence upon ammonia excretion. If it did have an influence upon ammonia excretion, one would expect he

TABLE 15

Intravenous injection of water. Data from second injection

TIME	GASTRIC JUICE			URINE		REMARKS
	Amount	Free acidity	Total acidity	Amount	NH_3 N	
a. m.	*cc.*			*cc.*	*mgm.*	
10.30	43	2.5	12.5			
10.45	7	22.5	35.0			
11.00	4	42.5	50.0	12.8	3.0	
11.15	7	35.0	45.0			
11.30	7	25.0	30.0	20.0	4.7	
11.45	3	22.0	30.0			
12.00	6	22.0	25.0	21.6	6.8	
12.15	7	20.0	25.0			
12.30	3	22.0	30.0	13.4	4.5	
12.45	4	27.0	35.0			
1.00	4	25.0	32.0	11.8	5.1	Injection began at 1.10 and was
Inj. 1.15						stopped at 1.29
1.30	27	22.0	32.0	11.0	5.0	Hemaglobinuria
1.45	10	30.0	40.0			
2.00	7	32.0	42.0	4.4	2.4	
2.15	8	27.0	40.0			
2.30	8	30.0	42.0	4.4	2.4	
2.45	5	32.0	42.0			
3.00	5	30.0	32.0	4.8	2.5	
3.15	4	20.0	25.0			
3.30	8	17.0	25.0	4.2	2.5	
3.45	4	20.0	27.0			Hemaglobinuria stopped at 7.30
4.00	6	25.0	30.0	3.2	2.0	

greatest amount of ammonia to be present during the period of greatest diuresis, which does not generally occur as can be verified by reviewing tables 1 to 10.

Intravenous injection of redistilled water: man

It has been reported (unpublished results) by Doctor Sutherland, working in this laboratory, that intravenous injection of water caused an increase in gastric secretion. With this in view and desiring to

note if injection of water into the blood stream influenced urine ammonia in any way, three intravenous injections of 200 cc. of redistilled water were made in one man. The injection[4] was made over a period of fifteen minutes, the needle being inserted into the basilic vein. The urine and gastric juice were collected for a period previous to the injection as a control. Table 15 shows the results obtained.

A slight gastric stimulation resulted upon the injection of the water (table 15). The urine amount as well as the urine ammonia was decreased. The acid gastric secretion was not allowed to pass into the intestine and no acid was absorbed, explaining the absence of any increase in urine ammonia. The gastric stimulation was so slight that it is doubtful that there would have been an increase in urine ammonia even if the juice had been absorbed.

GENERAL DISCUSSION

It is well known that the ammonia excretion is markedly dependent upon the balance of acids and bases in the body under physiological as well as pathological conditions. Whether or not there will be an increase or a decrease in urine ammonia during digestion will depend upon this acid-base balance. It must be kept in mind that during digestion, although acid is taken from the blood to form gastric juice, alkali is also used to form pancreatic juice. In fact Hasselbalch's (7) results show almost invariably a greater acidity of the blood, as judged from the H-ion concentration of the urine, for the period after the meal. One would expect the urine to become more alkaline if the blood became much more alkaline during the secretion of gastric juice. Higgins (8), repeating Hasselbalch's work, found "no marked difference between breakfastless and food periods." At any rate urine does not increase in alkalinity during digestion. However, Higgins (8) and Erdt (9) both found an increase in the alveolar CO_2 tension, the former assigning the increase to the slight change in condition rather than to any effect upon blood alkali, and the latter to an increase in the reserve alkali of the blood caused by the secretion of HCl. D. D. Van Slyke, Stillman and Cullen (10) found, by taking samples of blood before the

[4] A hemaglobinuria resulted upon injection and lasted five hours. It was apparent at the first urination and was most marked three-fourths of an hour after the injection ceased. The hemaglobin index was reduced from 92 to 84 (Haldane—Sahli method). The conductivity of the blood was increased slightly. No other reaction was observed.

meal and from one-half to two hours after the meal, "that the alveolar CO_2 tension rises after a meal" and that "plasma carbonate in some cases increases slightly, in others does not," results which, Van Slyke says, confirm Higgin's rather than Erdt's explanation of the increased alveolar CO_2 tension during digestion. These findings offer an explanation, then, for the variations in normal and pathological individuals and probably explain why in some of the subjects of my series no increase in urine ammonia occurred during digestion and absorption, for the greater the alkaline reserve or base balance of the blood, the less will be the urine ammonia, and vice versa, for a smaller or a greater amount of NH_3 would be called upon to act as "buffer" to the acid absorbed.

Since there is no marked change in the acid-base balance of the blood during digestion, it is apparent that the absorption of acid chyme would be followed by an increase in urine ammonia and that any factor that would increase the amount of acid in the chyme or increase the rate of absorption of the acid would be followed by an increase in urine ammonia. On the other hand, if this acid is neutralized before being absorbed, no increase in urine ammonia will occur. Whether neutralization of the acid outside the intestine by $NaHCO_3$, as was done in this study, is comparable to neutralization by the alkaline pancreatic juice in the intestine is a question. Nevertheless it seems obvious that neutralization of the acid chyme by the bases of the alkaline digestive juices, coupled with relatively slow absorption, would not place an extra task upon the acid-base balance of the blood and as a result no increase, or possibly a slight increase, in the urine ammonia would occur. (The diminution in ammonia after the injection of the neutral chyme in table 8 was probably due to the excess in alkalinity of the neutral chyme plus the alkaline digestive juices.) But if the acid chyme is thrown into the intestine at a faster rate,[5] and if its acidity and rate of absorption are increased as when the water-ingestion with the meals is increased, it is obvious that a noticeable increase in urine ammonia would occur for this sudden excess of acid would require ammonia for its neutralization in order to prevent the utilization of the plasma alkaline reserve or a change in the H-ion concentration of the blood.

[5] See table 1 and a later paper.

CONCLUSIONS

1. The ammonia excretion after the ingestion of a meal varies slightly in the same individual and markedly in different individuals. There was an increase in urine ammonia after the ingestion of a meal in the majority of cases studied in this series. A marked increase occurred in the dogs worked upon.

2. During gastric stimulation by food or by water followed by absorption in the intestine there is an increase in urine ammonia.

3. The degree of increase in urine ammonia upon the absorption of acid chyme is dependent upon the rate of absorption of the acid chyme or, in other words, its fluid consistency.

4. During gastric secretion not followed by absorption in the intestine no increase in urine ammonia occurs.

5 *a*. The absorption of water from the intestine (distal or proximal) causes some diuresis but no change in urine ammonia.

b. The absorption of alkali from the intestine causes diuresis with a marked decrease in urine ammonia.

c. The absorption of acid from the intestine causes some diuresis with an increase in urine ammonia.

6. Diuresis per se causes no change in urine ammonia.

7. Intravenous injection of water causes some gastric stimulation but no increase in urine ammonia or urine output.

So gastric secretion and urine ammonia are related in that the urine ammonia is increased by the absorption in the intestine of the acid product of gastric secretion, provided that this acid secretion is absorbed before neutralization occurs, i.e., at a relatively fast rate.

The writer desires to acknowledge his indebtedness to Doctors Luckhardt and Carlson for their valuable criticisms.

BIBLIOGRAPHY

(1) Hawk and Wills: Journ. Amer. Chem. Soc., 1914, xxxvi.
(2) Walter: Arch. Exper. Path., 1877, vii, 148.
(3) Schittenhelm: Deutsch. Arch. klin. Med., 1905, lxxvii.
(4) Loeb: Zeitschr. f. klin. Med., 1905, lvi, 100.
(5) Gammeltoft: Zeitschr. f. Physiol. Chem., 1911, lxxv, 66.
(6) Folin and Bell: Journ. Biol. Chem., 1917, xxix, 329; Ibid., 1916, xxvi, 473.
(7) Hasselbalch: Biochem. Zeitschr., 1912, xlvi, 403.
(8) Higgins: This Journal, 1914, xxiv, 114.
(9) Erdt: Deutsch. Arch. klin. Med., 1915, xlvii, 497.
(10) Van Slyke, Stillman and Cullen: Journ. Biol. Chem., 1907, xxx, 404.

THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

VII. Venous Discharge from the Adrenal Glands

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As has been pointed out before, adrenin possesses the selective action of stimulating the terminations of the sympathetic nervous system (1). Intravenous injections of adrenin then should duplicate the results of artificial stimulation of the sympathetics to a given tissue.

Of all the endocrine glands, the adrenals and their innervation have probably received the greatest attention at the hands of investigators. Jacobi (2) early described branches of the splanchnic nerves which pass into the adrenal glands and Dogiel (3) later established the anatomical relation of these nerves to the adrenals. The observation by Biedl (4) that stimulation of the splanchnic nerves produced vasodilatation in the gland was soon confirmed by Dryer (5), Tschboksaroff (6) and later by many other observers. The conclusion was that the splanchnics carry vasodilator fibers to the adrenal glands. G. von Anrep (7) in his study of the relations of the suprarenal glands to the normal vascular reactions of the body, observed dilatation to occur in the glands following the administration of adrenin by vein. Neuman (8) using massive doses of adrenin, observed that the blood flow through the adrenal glands was slightly increased during the pressor effect of an adrenin injection.

In the studies of the effect of adrenin on the circulation in the adrenal glands, short lasting injections of varying dosage only have been used. No records of infusions, which probably most nearly simulate the normal discharge of the glands, could be found. It was considered therefore that a careful study of both injections and infusions of varying dosage might conceivably give different results than those recorded.

METHOD

The method of investigation used in this study has been that described for the outflow in previous papers of the series (1). The left adrenal gland was exposed through median and lateral incisions in the abdominal wall and its peritoneal covering removed. The large vein from the abdominal wall which crosses the gland and pours into the adrenal vein was ligated about one inch distal to the gland. An oiled cannula was then inserted into the vein central to the ligature. The adrenal vein was then tied off close to the renal vein. The venous circulation in the short section of vein was thus reversed. This method was used simply for convenience and it interfered in no way with the normal venous discharge from the gland.

Dogs under ether anesthesia were used as experimental animals. The blood pressure was recorded from the femoral artery as before described (1).

RESULTS

The effects of adrenin ("adrenalin") in pressor, depressor and neutral injections and infusions were studied, the drug being injected into the femoral vein.

It was found that the effect of adrenin on the circulation in the adrenal glands was much less than the recorded literature on the subject would lead one to expect. Figure 1 shows the result of a pressor infusion. The only noticeable effect was a short lasting increase in the outflow which occurred during the early part of the rise in arterial pressure. This may probably be explained as due to the propulsive effect of the increased output from the heart. Its brevity strongly suggests that it is not due to a direct action of the drug on the gland vessels.

Figure 2 shows the effect of an injection which is at first pressor and then depressor. During the early part of the pressor stage there occurs the characteristic increase in outflow. During the depressor stage there is a diminution in the outflow. This, however, is only an apparent dilatation in the gland followed by constriction. On closer analysis the change in the outflow can be interpreted as entirely passive. During the whole of the blood pressure reaction the average rate of outflow was one drop per second. This is exactly the average rate of outflow before and after the injection. Regardless of the injection the rate of flow through the gland per minute was the same.

The action then of adrenin on the circulation in the adrenal glands is purely passive.

Fig. 1. Effects of an infusion of 20 cc. of 1–100,000 adrenin in two minutes and five seconds on the outflow from the suprarenal vein. Blood pressure manometer slightly damped. Dog weight, 11 kilos.

Fig. 2. Effects of 3 cc. of 1–100,000 adrenin on the outflow from the suprarenal vein. Blood pressure manometer damped. Dog weight, 10 kilos.

DISCUSSION

The trauma necessary to the experimentation can hardly be responsible for the observed results. The sympathetics pass to the glands along the suprarenal artery. In cannulating the adrenal vein, the arterial supply was carefully preserved. The failure of adrenin to produce any active change in the blood flow through the adrenal glands leads to the conclusion that the splanchnic nerves do not carry vasomotor fibers to the glands.

Since the completion of this work a paper by Burton-Opitz and Edwards (9) has appeared on the vasomotor supply to the adrenal glands which supports this conclusion. These investigators measured the blood flow through the glands with the stromuhr. They observed no change in the blood flow through the gland from splanchnic stimulation providing the general arterial pressure was maintained at a constant level by simultaneous stimulation of the central end of the splanchnics.

CONCLUSIONS

1. Intravenous injections of adrenin produce no essential changes in the blood flow through the adrenal glands.

2. The changes produced passively follow the general arterial pressure.

3. The splanchnic nerves do not carry vasomotor fibers to the adrenal glands.

BIBLIOGRAPHY

(1) Gunning: This Journal, 1917, xliv, 215.
(2) Jacobi: Arch. f. Exper. Path. u. Pharm., 1891, xxix, 185.
(3) Dogiel: Arch. f. Anat. u. Physiol., 1894, 90.
(4) Biedl: Pflüger's Arch., 1887, lxvii, 443.
(5) Dryer: This Journal, 1899, ii, 203.
(6) Tschboksaroff, Pflüger's Arch., 1910, cxxxvii, 59.
(7) von Anrep: Journ. Physiol., 1912, xlv, 307.
(8) Neuman: Journ. Physiol., 1912, xlv, 188.
(9) Burton-Opitz and Edwards: This Journal, 1917, xliii, 408.

THE ANTIGENIC PROPERTY OF CLOSED INTESTINAL LOOP FLUID

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Whipple, Stone and Bernheim (1), (2), (3), have pointed out the value of isolated closed intestinal loops in the study of some of the obscure features of acute intestinal obstruction. After varying intervals, depending on the location of the closed loop, there accumulates in these loops a substance which, upon injection, causes similar but more intense signs of intoxication than those in dogs with obstruction. The picture is one of severe toxemia with low blood pressure, low temperature, vomiting and diarrhea. It has been stated by these and many other workers that the toxic material in this obstructed portion contains the toxin or toxins of intestinal obstruction and, as a result, a great amount of work has been done to investigate the nature of this fluid. Whipple and his coworkers have concluded that the important toxic factor is of a proteose nature and have endeavored to show that it can provoke its specific antibodies on injection as well as increase the resistance of a normal dog to experimental obstruction. In a more extensive work (4) one of us, working with Doctor Moorhead, failed to corroborate this. The present study is in part an outgrowth of this work, being a further investigation of the problem on an animal much more suited for immunological study than the dog.

A study such as this is somewhat illogical at a time when so little is known of the chemical substances, which must surely be many and varied, present in the closed loop fluid. Inasmuch however as a study of this kind will throw light on this question of the chemical nature of the toxins of the loop fluid, it is justifiable and will be followed soon by comprehensive chemical and analytical studies.

The literature is not very conclusive as to the chemical nature of substances which may act as antigens. In general it is accepted that all proteins have, and all nonproteins do not have, antigenic properties. The question is by no means settled and involves a discussion of the

chemical nature of enzymes, the structure of animal and vegetable toxins, etc. See the discussions by Wells (5) and Pick (6). The best established exception is that of Ford's work on mushrooms and poison ivy in which he contends that the poisons are glucosides and nevertheless yield antibodies. Most other works err either in not having the antigen protein free or in confusing an inhibiting substance in the serum with a new formed antibody.

In addition to possible practical therapeutic application, the production of a specific immune body to the toxin or toxins present in acute intestinal obstruction would indicate that these toxins are probably proteins or the closely allied albumoses or proteoses; whereas a failure to produce any sort of immunological reaction after repeated injection with suitable doses in suitable hosts may be taken to point to a toxic substance of non-protein nature.

METHOD

The loop fluid was obtained from intestinal loops produced in the manner described by Dragstedt, Moorhead and Burcky (8). Isolated closed loops of the duodenum, duodeno-jejunum, or beginning jejunum, produced in this manner were found to contain after forty-eight hours from 80 to 100 cc. of a bloody, foul-smelling fluid. This fluid was strained through a coarse mesh to remove the fragments of sloughed mucosa, etc., and then heated for a hour over a water bath at 70°C. This resulted in the formation of a large coagulum which was filtered off. Such treatment was found not to affect the toxicity of the loop fluid. The filtrate was then kept between toluol and chloroform at room temperature. Before using, the preservatives were removed by further heating to 70°C. and the fluid again filtered. Injections were made into the marginal ear vein of healthy rabbits primarily at intervals of four days, later at varied intervals according to the progress of the animal.

The immunological work consisted of *a*, a study of the comparative resistance of normal rabbits and rabbits repeatedly injected with loop fluid, both as to the lethal dose and to the effect of equal doses on the blood pressure; *b*, a study of the serum of injected rabbits with reference to the appearance of any neutralizing or antitoxic substance; and *c*, observations regarding any indication of anaphylaxis.

a. Question of increased resistance after loop fluid injection. Two series of experiments were conducted with reference to this point.

In the first series the rabbits were injected with a dose slightly less than the lethal. This should result in a marked and rapid production of antibodies and the increased resistance could be easily studied by but slight variations in the amount injected; e.g., a rabbit that had received several injections of 2 cc. per kilo should easily resist a dose of say 2.5 cc. per kilo if there was any increased resistance. The fluid used was standardized by injection into five normal rabbits (see table 1).

TABLE 1

Standardization of fluid XO_2

RABBIT NUMBER	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
	grams	*cc.*		
2	1900	7.5	4.0	Dead in 6 hours
3	2800	7.8	2.8	Dead in 12 hours
4	2280	6.8	3.0	Dead in 4 hours
6	2160	4.3	2.0	Dead in 4½ hours
1	1440	4.3	3.0	Survived

It will be observed that there is a very considerable normal variation in the dose that is lethal to rabbits. This of course must be taken into consideration when interpreting results. Two rabbits were then selected and injected for a number of days with fairly large doses. The details of dosage and result are tabulated (see table 2).

Neither rabbit showed an increased but rather a decreased tolerance to subsequent injections. This result was not in the nature of an anaphylactic reaction or protein sensitization phenomenon although, as will be described elsewhere, the injection of a lethal dose of loop fluid results in a post-mortem picture simulating that of an anaphylactic death, the effect being very much like that obtained by Dale and Laidlaw (9) with the depressor substance B-iminazolylethylamin when injected into rodents.

The injection of such large doses resulted in so marked a loss of body weight that although the animals were apparently in good condition upon recovery from each injection, there may have been a greater loss in normal resistance than increase in specific resistance (if there can be any differentiation between the two) to the toxins of the loop fluid. The second series of rabbits were therefore injected with smaller doses over a longer period of time, with more than usual effort to keep them well nourished and in good condition.

As the loop fluid used in this series was different from that of the first series it was standardized by injection into five rabbits with the results as tabulated in table 3. Three rabbits were then injected for a number of days, the total number of immunizing doses being six. The seventh dose was a larger one designed to test the rabbits' resist-

TABLE 2

Repeated injection of fluid XO_2

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
		grams	*cc.*		
1	1	1440	4.3	3.0	Moderate depression. Recovery
	4	1340	4.0	3.0	Moderate depression. Recovery
	7	1300	3.4	2.6	Slight depression. Recovery
	11	1300	3.9	3.0	Dead in 8 hours
7	1	1650	4.0	2.5	Marked depression. Recovery
	4	1550	3.1	2.0	Slight depression. Recovery
	6	1500	3.0	2.0	Slight depression. Recovery
	11	1500	3.0	2.0	Slight depression. Recovery
	14	1520	3.3	2.2	Dead in 8 hours

TABLE 3

Standardization of fluid I

RABBIT NUMBER	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
	grams	*cc.*		
17	2100	1.0	0.5	No depression
14	1370	1.0	0.7	Moderate depression. Recovery
16	1420	3.6	2.5	Moderate depression. Recovery
18	1500	3.0	2.0	Dead in 22 hours
19	1300	3.9	3.0	Dead in 18 hours

ance for comparison with the normal rabbits in table 3. The procedure and result for each rabbit are detailed in table 4.

Here again the results indicate no increased resistance. The survival of rabbit 12 is well within the limit of normal variation as illustrated in tables 3 and 1.

The final experiment to test the possibility of an increased resistance in the "immunized" animal was a blood pressure experiment, comparing the effect of equal doses of loop fluid upon the blood pressure in a

normal rabbit with that upon the blood pressure of a rabbit repeatedly injected with loop fluid. Several experiments of this kind were done and while the results varied sometimes widely, there were never any variations that could be ascribed to an immunity or lack of immunity per se.

b. The question of a neutralizing substance in the serum of rabbits repeatedly injected with loop fluid. Although the normal variation in

TABLE 4

Repeated injection of fluid I

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	CUBIC CENTIMETERS PER KILO	RESULT
		grams	*cc.*		
11	1	1625	1.6	1.0	Slight depression. Recovery
	6	1600	1.6	1.0	Slight depression. Recovery
	13	1470	1.0	0.7	Slight depression. Recovery
	21	1300	0.6	0.5	Slight depression. Recovery
	31	1400	0.8	0.58	Slight depression. Recovery
	42	1500	1.0	0.7	Slight depression. Recovery
	55	1800	3.6	2.0	Dead in 24 hours
13	1	1100	1.1	1.0	Slight depression. Recovery
	7	1020	1.0	1.0	Slight depression. Recovery
	13	1080	0.7	0.7	Slight depression. Recovery
	20	1000	0.6	0.6	Slight depression. Recovery
	31	1180	0.8	0.7	Slight depression. Recovery
	42	1100	1.0	1.0	Slight depression. Recovery
	53	1200	3.0	2.5	Dead in 24 hours
12	1	1360	1.4	1.0	Slight depression. Recovery
	7	1240	1.0	0.8	Slight depression. Recovery
	13	1320	1.0	0.7	Slight depression. Recovery
	20	1140	0.6	0.5	Slight depression. Recovery
	31	1220	0.7	0.6	Slight depression. Recovery
	67	1440	1.0	0.7	Slight depression. Recovery
	83	1600	4.0	2.5	Marked depression. Recovery

resistance to loop fluid is very large in different rabbits (see tables 1 and 3) and it is therefore difficult to establish a definite minimum lethal dose, indicative results should surely be obtained if there is any definite neutralizing substance in the serum of rabbits repeatedly injected with loop fluid. Whipple, Stone and Bernheim (10) report negative results combining the serum from a dog that had been repeatedly in-

jected with large doses of loop fluid, with loop fluid of known toxicity and injecting the mixture. They however report that organ extracts and emulsions (liver, spleen, lung) of "immune" dogs rapidly destroy the loop poison during incubation in vitro. They conceive of an immunity residing in the tissue cells. This finding may be in accord with that of Kraus and Lipschutz (11), who report that the extracts of normal organs are richer in antitoxin against certain bacteriolysins than is the serum of the same animal. This point will be discussed later.

A rabbit that had been repeatedly injected with loop fluid (see table 5) was bled to death, the blood defibrinated, centrifuged and a clear serum obtained. Loop fluid "I" was used which was standardized so that 2 cc. per kilo was the approximate minimum lethal dose (see table

TABLE 5

Procedure with rabbit whose serum was tested

RABBIT NUMBER	DAYS	WEIGHT	AMOUNT INJECTED	REMARKS
		grams	*cc.*	
9	1	1800	0.9	Slight depression. Recovery
	7	1600	1.0	Slight depression. Recovery
	14	1490	1.2	Slight depression. Recovery
	22	1340	0.5	Slight depression. Recovery
	32	1420	0.7	Slight depression. Recovery
	43	1400	1.0	Slight depression. Recovery
	61	1400		Bled to death

3). Two normal rabbits were injected, one with a mixture of a loop fluid and serum (equal parts) that had been kept two hours in the incubator at 38°C., the other with a mixture that had remained two hours in the incubator and then kept over night in the ice box. Both animals died in less than twenty-four hours with symptoms and post-mortem findings the same as when no serum had been used (see table 6).

A similar experiment was done on the guinea pig. The injection of about 1 cc. of loop fluid intraperitoneally into a 350 gram guinea pig caused death in twelve to fourteen hours with symptoms like those in anaphylactic shock, respiratory difficulty, etc. Combining the loop fluid with the serum obtained above in no way altered either the amount necessary to cause death or the symptoms and autopsy findings.

A further test was made to see if the serum used above could destroy the depressor effect of loop fluid on the blood pressure of a dog. The

serum was incubated with the loop fluid for two hours and the mixture then kept over night in the ice box. In several such experiments no effect of either neutralization or augmentation of the depressor effect could be noticed.

c. Observations regarding anaphylaxis. During the course of the experiments no indications of anaphylaxis were observed. This was the case in the series of rabbits that were repeatedly injected for the experiments above and also in three other rabbits that received sensitizing doses of 0.25 cc. loop fluid and test doses of 1 cc., eleven days later. The symptoms after the injection of large doses of loop fluid into rodents are very much like those seen in anaphylactic shock, however. This is also recorded as the result of the injection of B-iminazolylethylamine by Dale and Laidlaw (9). This substance Barger and Dale have shown to be present in intestinal mucosa (12),

TABLE 6

Injection of mixture of rabbit's serum and loop fluid

RABBIT NUMBER	WEIGHT	CUBIC CENTIMETERS LOOP FLUID	CUBIC CENTIMETERS SERUM	TREATMENT OF MIXTURE	RESULT
	grams				
20	2000	4	4	2 hours at 37°C.	Dead in 22 hours
21	1450	2.5	2.5	2 hours at 37°C., overnight in ice box	Dead in 18 hours

an observation which indicates that this interesting substance may be the important constituent of closed intestinal loop fluid. Experiments are at present being done to further compare the physiological action of loop fluid with that of B-iminazolylethylamine.

The absence of any anaphylactic phenomena indicates that we are not dealing with toxic substances of protein or proteose nature. (See Gay (13) and Wells and Osborne (14).)

No test tube experiments, complement fixation or serum reactions, etc., were performed because here no helpful conclusions could be drawn from such observations. If there were no visible reactions upon adding "immune" serum to the test loop fluid it would not indicate that there was neutralization or interaction between the two as a visible reaction is not a sine qua non to such interaction. On the other hand if there were phenomena of precipitation, etc., we could not conclude

that there was a reaction between the "immune" serum and the toxic principle as the latter is present in such a composite fluid that any number of results are possible.

DISCUSSION

At no time during the course of these experiments was there any indication of a definite immunity being established in rabbits following the repeated injection of closed intestinal loop fluid. The very large variation in resistance to the injection of this toxic material that is met with in normal rabbits can adequately account for all seeming instances of immunity in our opinion.

The experiments of Whipple and his coworkers in "autolyzing" loop fluid with organ extracts and observing a decrease in toxicity as a result cannot be considered as demonstrating an immune reaction. Ewins and Laidlaw (15) have found that p-hydro-xyphenylethylamine is readily converted into p-hydro-xyphenylacetic acid by the perfused rabbits liver and also to some extent by the perfused isolated uterus, while if the isolated heart is perfused the amine is completely destroyed. They also demonstrated that indolethylamin is converted to indoleacetic acid by the perfused liver (16). More recently Guggenheim and Loffler (17) have made a more comprehensive study of the fate of proteogenic amines in the body. They corroborated the work of Ewins and Laidlaw and demonstrated that a large number of the very toxic amines are rapidly detoxicated after introduction either orally or intravenously. It was demonstrated by perfusion of the liver that this organ can detoxicate these substances by deaminisation and oxidation of the amine to the corresponding carboxylic acid. It is very likely that this detoxication is in part a general tissue reaction and is the phenomenon responsible for the loss of toxicity observed by Whipple et al after incubating the toxic loop fluid with organ extracts.

Davis and Stone (18) have recently shown that normal intestinal secretion is non-toxic upon intravenous injection. This was to be expected inasmuch as dogs show no intoxication after the production of open intestinal loops that are permitted to drain into the abdominal cavity (4), (9). Such secretion, however, when kept free from preservatives and unheated, rapidly becomes toxic producing the same effects upon intravenous injection as closed loop fluid. A rapid and profuse growth of bacteria in this secretion was noted, and while they do not consider it conclusively demonstrated that bacteria are responsible for the development of this toxicity, it is certain that the end products of bacterial activity are concerned.

CONCLUSIONS

1. There are no specific antibodies produced following the repeated intravenous injection of closed loop fluid in rabbits.

2. The toxic principles of closed loop fluid are probably not of protein nature.

The authors wish to acknowledge the helpful criticism of Dr. Preston Kyes.

BIBLIOGRAPHY

(1) Stone, Bernheim and Whipple: Bull. Johns Hopkins Hosp., 1912, xxiii, 159.
(2) Whipple, Stone and Bernheim: Journ. Exper. Med., 1913, xvii, 286.
(3) Whipple, Stone and Bernheim: Journ. Exper. Med., 1913, xvii, 307.
(4) Dragstedt and Moorhead: Journ. Exper. Med., 1918, xxvii, 359.
(5) Wells: Chemical pathology, 2nd Ed., 1914, 156
(6) Pick: Wassermann's Handb. d. path. Mikroörganismen, 1912, i, 685.
(7) Ford: Journ. Infec. Dis., 1907, iv, 541.
(8) Dragstedt, Moorhead and Burcky: Journ. Exper. Med., 1917, xxv, 421.
(9) Dale and Laidlaw: Journ. Physiol., 1910, xli, 318.
(10) Whipple, Stone and Bernheim: Journ. Exper. Med., 1914, xix, 144.
(11) Kraus and Lipschütz: Zeitschr. f. Hyg., 1904, xlvi, 49.
(12) Barger and Dale: Journ. Physiol., 1911, xli, 499.
(13) Gay: Trans. Assoc. Amer. Physicians, 1913, xxviii, 13.
(14) Wells and Osborne: Journ. Infec. Dis., 1915, xvii, 259.
(15) Ewins and Laidlaw: Journ. Physiol., 1910, xli, 78.
(16) Ewins and Laidlaw: Biochem. Journ., 1913, vii, 18.
(17) Guggenheim and Loffler: Biochem. Zeitschr., 1916, lxxii, 325.
(18) Davis and Stone: Journ. Exper. Med., 1917, xxvi, 687.

STUDIES ON THE THROMBOPLASTIC ACTION OF CEPHALIN

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HISTORICAL

A great deal of light has been thrown recently upon the process of coagulation of the blood and the part played by the different blood constituents in this process. All the various theories on the normal blood coagulation have been recently reviewed by Morawitz (1), Whipple (2) and others.

The theory of Howell (3), (4) on the effect of tissue juices in neutralizing the antithrombin content of normal blood has been also reviewed by a number of investigators. It may be worth while to state briefly this theory, so as to keep in mind the different factors which play a part in normal blood coagulation. The normal plasma contains prothrombin and calcium which go to make up thrombin, the ferment which acts upon the fibrinogen of the blood and converts it into fibrin or the normal blood clot; but normal blood contains also an antithrombin which binds the prothrombin and renders it inactive and before the prothrombin can become active, the antithrombin has to be neutralized by thromboplastin derived from different cells. Howell (5) has also isolated the lipoid cephalin from the brain tissues and has shown that this substance has strong thromboplastic properties in neutralizing the antithrombin present in normal blood and thus allowing the blood to clot rapidly. Hammarsten (6), basing his claim on his own work and that of others, stated that only the calcium precipitated out of the blood by means of a soluble oxalate is necessary to transform the fibrin ferment from the inactive into the active form; the second phase of the process of blood clotting, namely the action of thrombin upon the fibrinogen, can take place also in the absence of calcium salts.

Without going into a detailed discussion as to the different theories on blood coagulation and as to which of them has more scientific evi-

dence in explaining this phenomenon, we will find one thing to hold true and that is that a substance can be obtained from different tissues which has a strong thromboplastic action, greatly accelerating the clotting of the blood. This substance, cephalin, has been isolated by Howell, McLean and others from the brain and other tissues of the body. To test the thromboplastic action of cephalin Howell (5) used a peptone plasma obtained by injecting a solution of Witte's peptone into a dog, 0.4 to 0.5 gram to each kilogram of animal weight; the clear plasma obtained by centrifugalizing the blood of the dog was evaporated to dryness; small quantities of the evaporated plasma were rubbed up with physiological salt solution, filtered and used for the test. The thromboplastic substance was obtained by Howell (5) by extracting dried brain tissues with ether, evaporating the ether, then precipitating the cephalin with acetone and washing with alcohol. A solution of this substance will neutralize the action of the antithrombin of the blood. In another paper Howell (4) reviews the different hypotheses as to the nature of the thromboplastic substance extracted from the tissues and furnishes evidence to show that it facilitates the clotting of the blood only in those plasmas in which antithrombin is present and that it acts by neutralizing this antithrombin.

McLean (7) further reports a method of testing the thromboplastic activity of cephalin; it consists in activating the ineffective thrombin present in fresh serum in relatively large amounts. When to eight drops of oxalated plasma three drops of 0.1 per cent cephalin solution in distilled water and three drops of fresh serum are added, a solid clot is produced in about one minute while the control clots only in thirty minutes to two hours. McLean (8) has further demonstrated the fact that cephalin exposed to the atmosphere or even kept in a desiccator over $CaCl_2$ gradually loses its thromboplastic properties, due to the fact that the unsaturated group of cephalin becomes saturated. Saturated or partly saturated cephalin not only will not exert a thromboplastic action, but a solution of it will give an acid reaction and even retard the coagulation of the blood.

Clinical studies by different investigators have shown that cephalin exerts a very strong hemostatic action when applied to wounds; reference can be made here to the work of Hirschfelder (9) and Horwitz (10).

EXPERIMENTAL

The method of preparation of cephalin was the same, with slight modifications, as that used by Howell and McLean for the preparation of their laboratory cephalin. Brains obtained from different animals were cleaned of all the membranes and blood vessels, macerated to a pulp, spread on plates and dried in a current of warm air for twenty-four to forty-eight hours; the dried residue was then extracted with ether for twenty-four hours; the ether solution of cephalin was removed and the residual mass extracted with another quantity of ether for twenty-four hours. Both extracts were filtered and the filtrates evaporated in a current of cold air to a semisolid residue; the latter was precipitated and washed several times with acetone. The precipitate was then redissolved in ether and filtered. The filtrate was evaporated nearly to dryness, then placed in a desiccator over sulfuric acid where the cephalin was freed from the last traces of ether and acetone without exposing it too much to the action of the air in the last process. The cephalin was then placed in amber glass bottles, evacuated and sealed off.

To test the different cephalin preparations and the effect of the environmental conditions upon this thromboplastic substance, the method used by Howell and McLean was followed. Tubes having a flat bottom, three-quarters of an inch in diameter and two inches high, were used throughout the work for all comparative studies; the observations were carried out at room temperature at about 17° to 19°C., except when otherwise stated. Complete invertibility of the clot was taken as the end point of the reaction. The procedure was carried out as follows: the desired amount of plasma (10 drops used in all cases) was first introduced into the thoroughly cleaned tubes using one clean pipette to fill all the tubes in the experiment; enough distilled water was then added with another clean pipette so that all the tubes, after the addition of the other ingredients, should contain the same quantity of fluid. The cephalin (1 per cent except when otherwise stated) was then introduced with a fresh pipette and soon after the serum or the $Ca(OH)_2$, when used. The introduction of the different constituents into the tubes was done as quickly as possible and time taken at the addition of the cephalin and serum or cephalin and $Ca(OH)_2$.

Difficulties were first experienced in obtaining a good supply of easily available plasma free from any large excess of anticoagulant. The dialyzing of the plasma, particularly in the presence of a large

excess of oxalate, did not give an absolutely oxalate free plasma. The use of fresh blood drawn from an animal with a paraffined syringe into paraffin coated containers, as suggested by Morawitz and Bierich, was first tried but this could not supply a material easily available at all times. It was therefore decided to find out just what concentration of sodium oxalate or sodium citrate was necessary to prevent the clotting of the blood, without any large excess of the salt being present in the plasma.

TABLE 1

The effect of the concentration of the anticoagulant upon the clotting of horse blood
Reading taken after 48 hours

ANTICOAGULANT	QUANTITY	CONCENTRATION	CLOT FORMATION
	grams	*per cent*	
Sodium oxalate	0.05	0.01	+
	0.10	0.02	+
	0.15	0.03	+
	0.20	0.04	+
	0.25	0.05	+
	0.30	0.06	−
	0.35	0.07	−
	0.40	0.08	−
	0.50	0.10	−
Sodium citrate	0.50	0.10	+
	1.00	0.20	*
	1.25	0.25	−
	1.50	0.30	−
	1.75	0.35	−
	2.00	0.40	−

* None first day; coagulation begins only after the first 20 to 25 hours.

Five hundred cubic centimeter quantities of horse blood were drawn into sterile bottles containing different quantities of sodium oxalate or sodium citrate and the clotting of the blood noted; the data are presented in table 1.

It is thus seen from the above table that 0.06 per cent of sodium oxalate or 0.2 to 0.25 per cent of sodium citrate is necessary to prevent the clotting of the blood and keep it unclotted for at least several days, particularly when the plasma is kept in the ice chest, thus supplying an easily available material without any of the difficulties experienced in the process of dialyzing the blood or the peptone bleeding, as used by Howell, and not introducing a large excess of anticoagulant.

By comparing the oxalated and the citrated plasma for the testing of the thromboplastic activities of the cephalin, the results obtained with citrated plasma were more definite and the action of the cephalin more pronounced, due to the fact that the excess of citrate still present in the plasma was more easily neutralized by the calcium introduced than the excess of the oxalate.

To standardize the method still further and perhaps throw some light upon the nature of the action of cephalin, it was decided to substitute in place of a fresh normal serum a standard solution of calcium hydroxide. As was shown above, some authors claim that the thromboplastic action of cephalin consists in activating the prothrombin present in the fresh serum and in the plasma. But in the absence of any fresh serum, the action of cephalin will have to be directed only toward neutralizing the antithrombin or inactivating the prothrombin

TABLE 2

The use of freshly drawn blood

SHEEP BLOOD	CONCENTRATION OF CEPHALIN, 2 DROPS	CLOT FORMATION
	per cent	*minutes*
10 drops	0.2	8
	0.02	14
	0.01	27
	0.002	26
	Water	43

of the plasma alone, if a sufficient amount of calcium is present to neutralize the excess of anticoagulant Hurwitz (10) emphasizes the fact that only fresh serum should be used; on standing a few days the thrombin of the serum is converted into an inactive form—metathrombin—so that old serum contains less thrombin and more antithrombin. Rich (11) has recently shown that metathrombin is a thrombin-antithrombin compound and is readily formed in solutions containing both thrombin and antithrombin.

The whole blood was used only in a few preliminary experiments, one of which is reported in table 2, and since the plasma containing the citrate or oxalate was found to be preferable, the latter has been used in all the other experiments.

The effect of the thromboplastic substance is well shown in the above table; even as low a concentration as 0.002 per cent stimulated

the coagulation of the blood; and increase in concentration resulted in an increase in the rapidity of coagulation.

TABLE 3

The influence of the amount of anticoagulant present in the plasma upon the action of cephalin

	ANTICOAGULANT	CEPHALIN FROM PIG'S BRAINS, 5 DROPS		
		Fresh horse serum	Clot formation	
			Control	Cephalin
	per cent	*drops*	*minutes*	*minutes*
Sodium citrate	0.2	3	240	5
	0.2	6	80	4
	0.2	10	14	3
	0.3	3	720	13
	0.3	6	120	8
	0.3	10	16	4
	0.4	6	240	45
	0.4	10	60	15
	0.5	6	More than 48 hours	120
	0.5	10	Imperfect clot in 24 hours	40
Sodium oxalate	0.07	3	24 hours	9
	0.07	6	15 hours	6
	0.07	10	5 hours	5
	0.1	3	Imperfect clot in 23 hours	48
	0.1	10	8 hours, 20 minutes	22

The results presented in table 3 clearly demonstrate the fact that the concentration of the anticoagulant has a decided effect upon the action of the cephalin, as a larger amount of serum has to be added to compensate for the presence of the excess of anticoagulant. This would tend to indicate that the addition of serum is not only for the purpose of supplying an inactive thrombin to the plasma-cephalin mixture but also in supplying a substance which neutralizes the anticoagulant.

To obtain further information on the possible rôle of the serum in neutralizing the anticoagulant of the plasma, a saturated solution of calcium hydroxide was substituted in place of serum.

The relative quantities of anticoagulant and calcium are found to have an important bearing upon the rapidity of coagulation. This confirms the observations made in table 3 that the calcium part of the

TABLE 4

*The neutralization of the excess of anticoagulant in plasma by calcium hydroxide**

	ANTICOAGULANT	$Ca(OH)_2$	CEPHALIN FROM OX'S BRAIN, 5 DROPS	
			Clot formed in minutes	
			Control	Cephalin
	per cent	*drops*		
Sodium citrate	0.20	3	30	6
	0.25	3	74	9
	0.27	3	170	29
	0.30	3	360	48
	0.35	3	430	52
Sodium oxalate	0.06	3	360	20
	0.07	3	380	30
	0.08	3	Over four days	75
Sodium citrate	0.2	1	62	8
	0.2	2	44	7
	0.2	3	26	6
	0.2	4	37	7
	0.2	5	72	7½
	0.2	6	117	8
	0.2	7		9
	0.2	8		18

* The calcium hydroxide solution used in this as well as in the following experiments was obtained by shaking some C. P. calcium hydrate with distilled water for five minutes, and then filtering.

serum neutralizes the anticoagulant, but this seems to be not the only function of the serum: while an increase in concentration of the serum always results in a more rapid coagulation, the same thing does not hold true with the calcium hydroxide. A further increase in concentration of calcium hydroxide, above the optimum, results in a delay in the rapidity of coagulation; this bears out the observations of Addis (12)

and Morawitz (13) that an excess of calcium may delay the coagulation of the blood. The more rapid coagulation resulting from an increase in the concentration of the serum may be therefore explained by the assumption that the calcium optimum has not been yet introduced with the smaller concentration of the serum or, what seems to be more probable, an increased amount of serum will introduce a larger amount of prothrombin which, after it has been activated by the cephalin or after the antithrombin has been neutralized by the cephalin, is converted into thrombin, which will act upon the fibrinogen of the plasma.

TABLE 5

The effect of the concentration of cephalin in accelerating the clotting of blood plasma

CONCENTRATION OF CEPHALIN IN WATER	CLOT FORMED IN MINUTES		DROPS OF 1 PER CENT ETHER SOLUTION	CLOT FORMED IN MINUTES SERUM USED
	Serum	$Ca(OH)_2$		
per cent				
1.0	10	10	1	10
0.8	8	9	2	12
0.6	10½	9	3	13
0.5	8½	11	4	10
0.4	9	11	5	10
0.3	9½	11½	6	13
0.25	12	12	7	12
0.20	13	13	8	12
0.15	12	14	9	12
0.10	15	14	10	10
0.05	18	15		
Control	280	180	Control	230

It was next thought advisable to study the effect of the concentration of cephalin upon its thromboplastic activities. A fresh lot of cephalin prepared from ox brain was dissolved in water so as to make up different concentrations. Horse blood plasma containing 0.2 per cent sodium citrate was distributed in tubes in the usual manner, five drops of fresh horse serum and five drops of the different concentrations of cephalin were added to these; to another series of tubes three drops of calcium hydroxide solution were added in place of the serum; to a third set of tubes a 1 per cent ether solution of cephalin was added.

Several interesting observations can be made from the above table. First of all the amount of a 1 per cent ether solution of cephalin does not seem to play any appreciable rôle in the rapidity of coagulation.

It looks as if enough cephalin has been introduced with one drop of the particular ether solution to produce the coagulation; the further increase may therefore not have any appreciable effect upon the rapidity of coagulation or may even retard it, perhaps by keeping the cephalin in the ether solution and not allowing it to act upon the mixture of serum and plasma. In the case of the water solution of cephalin there does not seem to be any difference between a 1.0 per cent solution and a 0.3 per cent solution, while in the case of the calcium hydroxide the rapidity of coagulation of mixtures to which five drops of a 1.0 per cent and a 0.2 per cent cephalin solution were added was the same;

TABLE 6

The effect of concentration of cephalin upon the rapidity of clotting of plasma

Ten drops of plasma containing 0.25 per cent sodium citrate; 3 drops calcium hydroxide solution, 1 per cent cephalin solution.

Cephalin drops	0	1	2	3	4	5	6	7	8	9	10
Distilled water drops	10	9	8	7	6	5	4	3	2	1	0
Clot formation in minutes	80	25	18	15	14	10	9	9	10	10	9

TABLE 7

The effect of concentration of cephalin upon the rapidity of clotting of plasma

Ten drops of plasma containing 0.25 per cent sodium citrate, 5 drops of fresh horse serum; 5 drops of fresh cephalin solution

	CONCENTRATION OF CEPHALIN											
Per cent	1	0.5	0.25	0.20	0.15	0.10	0.05	0.04	0.03	0.02	0.01	0.00
Clot formation in minutes	3	4	4	6	7	9	12	16	18	22	45	75

the further dilution gave in both cases a delayed coagulation. The conclusion made from this experiment would be that the concentration of the cephalin is not of great importance in the process of coagulation, if it does not fall below a certain concentration. Above that an increase in the concentration of the cephalin will not result in any further increase in the rapidity of coagulation, while a decrease in concentration will result in a decrease in the rapidity of coagulation. This concentration seems to fall between 0.25 and 0.30 per cent of cephalin solution in water of the particular lots tested when five drops of the solution and ten drops of plasma were used.

To obtain further information on this optimum concentration of cephalin and also on the time-concentration curve, the same experiment was repeated using lower dilutions of cephalin than in table 5. The data obtained by the use of an old lot of cephalin and three drops of calcium hydroxide are given in table 6; the use of very low dilutions and a fresh lot of cephalin are given in table 7.

The data presented in tables 6 and 7 confirm the previous observations. The rapidity of clotting does not decrease appreciably down

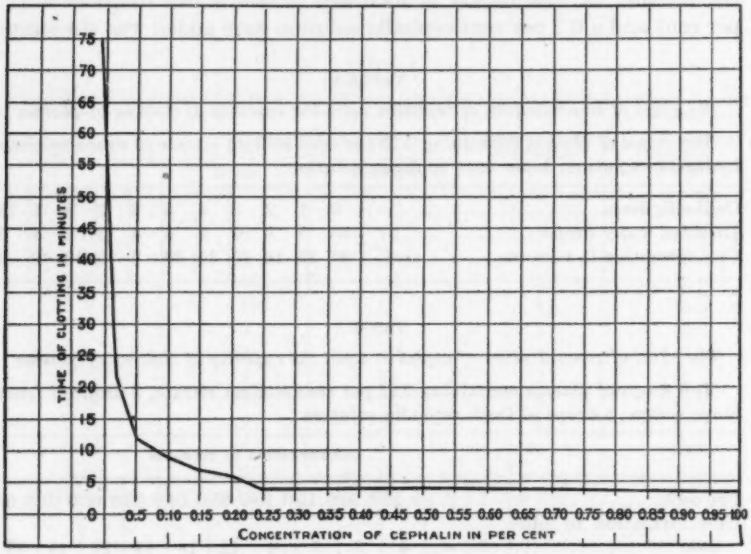

Fig. 1. The effects of concentration of cephalin upon the rapidity of clotting of plasma

to a concentration of 0.25 per cent of cephalin in water; on a further decrease in concentration the time of clotting rapidly increases.

Figure 1 shows the relation between the concentration of cephalin and the clotting time of the plasma; the data given in table 7 were used in plotting out the curve.

McLean (8) has shown that the thromboplastic action of cephalin deteriorates with age, particularly on exposure to air and to light, as indicated by its coagulating power and iodine absorption number. These observations were repeated in order to devise a method of keep-

ing the cephalin, especially when prepared in large quantities for the market, with the least deterioration of the material. The advisability of keeping the cephalin in the dark and in vacuated, sealed-off containers at once suggested itself. The vacuated cephalin samples used for this experiment were kept only in a partial vacuum, at about twenty-eight inches obtained by the use of the water pump. The results confirm fully McLean's observations; even an imperfect vacuum proved to be very favorable to the keeping properties of cephalin.

The exposure of the cephalin to the air seems to have an appreciable effect in lowering its thromboplastic properties. The vacuum-kept cephalin was in all instances superior to the lots exposed to the air. No doubt there are slight differences in the thromboplastic properties of the different cephalin preparations even when prepared from the brain of one particular animal, probably due to the fact that since the cephalin is not chemically pure, the impurities and the method of preparation may have a slight bearing upon it The cephalin prepared from the brains of the ox, sheep and pig does not differ greatly in its thromboplastic activities and the differences that do exist may be due to the slight differences in age of the preparations and in the method of keeping, either exposed to the air or evacuated, rather than to their origin.

The observations of McLean (8) are hereby confirmed: cephalin kept in loosely stoppered containers, in the presence of air, will deteriorate but this deterioration will be found by the method used in the previous experiments only when the cephalin is several months old. It was important to find out next how quickly a cephalin water solution will deteriorate.

One per cent solutions of different cephalin preparations were made up and allowed to stand in loosely stoppered glass containers at room temperature. At the end of forty-eight hours and seven days this solution was compared with a freshly prepared solution of the same lot of cephalin, using horse plasma containing 0.25 per cent sodium citrate, 5 drops of fresh horse serum, 3 drops of calcium hydroxide solution and 5 drops of the cephalin solution.

The activity of cephalin seems to diminish rapidly when kept in a water solution for any length of time. This is particularly true with an old lot of cephalin kept under unfavorable conditions. The fact was observed a number of times that fresh cephalin preparations which had a strong thromboplastic power deteriorated only very slowly in solution and often no deterioration was found in solutions forty-eight

TABLE 8

The influence of age of cephalin and method of keeping upon its thromboplastic action

CEPHALIN PREPARATION	FRESH SERUM	CEPHALIN	$Ca(OH)_2$	CLOT FORMATION
	drops	*drops*	*drops*	*minutes*
Ox's brain, fresh	5	5		5
	3	5		6
	2	5		9
	5	1		7
		5	3	8
Ox's brain, 3 months old, kept in vacuo	5	5		4
	3	5		4
	2	5		4½
	5	1		10
		5	3	4
Ox's brain, 6 months old, exposed	5	5		6
	3	5		8
	2	5		11 imperfect clot
	5	1		12
		5	3	9
Sheep's brain 1 month in desiccator, 2 months in vacuo	5	5		6
	3	5		8
	2	5		8
	5	1		10
		5	3	7
Sheep's brain, 16 months old, exposed to air	5	5		7
	3	5		8
	2	5		9
	5	1		13
		5	3	8
Pig's brain, 2½ months in vacuo	5	5		4
	3	5		5
	2	5		6
	5	1		5
		5	3	5
Control	5			250
	3			Over 12 hours
	2			Over 18 hours
			3	95 minutes

hours old, while in old lots of cephalin which exerted a relatively low thromboplastic action when kept in solution for only twenty-four to forty-eight hours, deterioration set in rapidly. Different cephalin preparations were kept in a water solution for over two weeks and tested every once in a while for their thromboplastic action; it was found that a deterioration set in only in the first few hours, but later the deterioration is less noticeable and, although weaker in their action than the freshly prepared solutions, they still exerted a marked effect in accelerating the coagulation of the blood.

TABLE 9

The influence of age of cephalin solution upon its thromboplastic properties

CEPHALIN PREPARATION	FRESH CEPHALIN SOLUTION		CLOT FORMATION IN MINUTES	CEPHALIN SOLUTION 48 HOURS OLD		CLOT FORMATION IN MINUTES
	Serum	$Ca(OH)_2$		Serum	$Ca(OH)_2$	
Ox's brain fresh	*		5	*		11
Ox's brain fresh		*	8		*	9
Ox's brain vacuated for 3 months	*		4	*		7
Ox's brain vacuated for 3 months		*	4		*	5
Pig's brain vacuated for 2½ months	*		4	*		9
Pig's brain vacuated for 2½ months		*	5		*	8
Ox's brain, 11 months in loosely stoppered container	*		7	*		20 imperfect clot
Control	*		90			
Control		*	72			

All the previous tests were conducted at room temperature. To study the effect of temperature upon the action of cephalin, particularly since its action upon the blood of animals would take place at the temperature of the body, two sets of tests were conducted at room and incubator (37°) temperature.

The effect of a higher temperature is thus found to be very beneficial in the process of coagulation of the blood by the use of cephalin. Since the phenomenon of coagulation, furthered by the introduction of cephalin, whether it consists in the neutralization of the antithrombin

or in stimulating the production of thrombin, is an enzyme phenomenon, we would expect that the action will take place more rapidly at a higher temperature.

The question of the nature of cephalin action and the part played by the presence of serum, in addition to the plasma and cephalin, is an interesting one. According to Howell's theory, the cephalin neutralizes the antithrombin thus allowing the calcium to activate the prothrombin and convert it into thrombin which acts upon the fibrinogen of the blood. The $Ca(OH)_2$ added will therefore neutralize the excess of citrate or oxalate present and allow the prothrombin of the plasma which became free to be converted into thrombin and after the cephalin acted upon the antithrombin and neutralized it, to act upon the fibrinogen and convert it into fibrin. The addition of serum to the plasma

TABLE 10

The effect of temperature upon the thromboplastic action of cephalin

Ten drops of plasma (0.25 per cent sodium citrate), 5 drops of serum or 3 drops of $Ca(OH)_2$ and 2 drops water, 5 drops of fresh cephalin (from ox brain) in different concentrations in water.

	CONCENTRATION OF CEPHALIN						CONTROL	
	0.5 per cent		0.2 per cent		0.1 per cent			
	18°	37°	18°	37°	18°	37°	18°	37°
Serum	4	2	6	4	9	7½	300	180
$Ca(OH)_2$	3½	1¼	5½	4	14	5	78	41

should therefore increase the amount of available prothrombin and allow the plasma to coagulate more rapidly, but this did not hold true in many cases, as seen in the previous experiments, where the plasma was coagulated almost as rapidly, if not more rapidly, in many instances, when $Ca(OH)_2$ was added in place of serum.

In the following experiments a fresh 1 per cent solution of cephalin was used for the study of the action of cephalin upon plasma, in the absence and in the presence of serum.

The calcium hydroxide seems to act upon the plasma only by neutralizing the excess of citrate, thus allowing the prothrombin in the plasma to be converted into the thrombin which acts upon the plasma and coagulates it; it seems to play no further rôle in the process of coagulation. A further increase in the concentration of the calcium above the amount necessary for the neutralization of the excess of

citrate is unnecessary, as is seen from table 12 where, in the presence of three drops of cephalin, three drops of Ca $(OH)_2$ exerted no more action than one drop, two drops of the solution used seem to be enough to just neutralize the excess of citrate and allow the cephalin to act upon the plasma. A further increase in the concentration of the calcium delays the action of the cephalin, as brought out in tables 4 and 12. This deleterious action of the excess of calcium can be neutralized by the addition of serum; the excess of free calcium probably combines with the proteins or salts of the serum, thus allowing the clotting to proceed in a normal manner.

TABLE 11

The influence of cephalin and serum upon the clotting of blood plasma

SERUM	CLOT FORMATION		
	No cephalin	Cephalin 1 drop	Cephalin 5 drops
drops		*minutes*	*minutes*
1	32 hours	25	6
2	26 hours	11	6
3	11 hours	15	5
4	6 hours	10	5
5	1 hour 30 minutes	10	5
6	42 minutes	8	4½
7	38 minutes	8	4½
8	40 minutes	7	3½
9	30 minutes	7	3½
10	18 minutes	7	3
No serum		30	12
No serum	Not clotted in 48 hours		

In adding serum to the plasma we seem to be introducing two different factors, one, probably the action of the calcium of the serum upon the excess of citrate of the plasma, and the other, exerting by itself a thromboplastic action upon the fibrinogen of the plasma, either supplying more prothrombin, which becomes active after the antithrombin has been neutralized and which is converted into thrombin due to the action of cephalin, or perhaps due to some other cause. That the action of serum is due to more than one factor is clearly seen from the fact that, by increasing the amount of serum, we increase the rapidity of coagulation. If the serum acted only by its calcium content there should be a maximum reached, above which a further addition of the serum would be without any effect; but as observed in table 11,

the increased addition of serum in the absence of cephalin to the plasma, gradually increased the rapidity of coagulation while, where cephalin has been added, the further increase of serum above a certain concentration does not increase, or only to a very small extent, the rapidity

TABLE 12

The influence of cephalin and $Ca(OH)_2$ upon the clotting of blood plasma in the absence and in the presence of serum

$Ca(OH)_2$	SERUM	CEPHALIN	CLOT FORMATION IN MINUTES
drops	*drops*	*drops*	
3		0	120
1		3	4
2		3	3½
3		3	4
3		1	9
3		5	4
0		1	32
0		2	13
0		3	15
0		4	15 imperfect clot
0		5	15 imperfect clot
0		6	10
0		7	7
0	5	1	6
0	5	5	4
0	1	3	7½
1	1	3	5
1	3	3	4
1	5	3	4
2	1	3	3½
2	3	3	3½
2	5	3	3½
3	1	3	3½
3	3	3	3
3	5	3	2¾
8	0	3	18
8	1	3	7
8	5	3	5

of coagulation. This would indicate that the serum, besides the introduction of calcium, also introduces small amounts of prothrombin, which although the antithrombin has not been neutralized, is able to act in increasing amounts upon the fibrinogen of the plasma. When an excess of cephalin is introduced, enough thrombin is formed or liberated to produce an optimum action upon the fibrinogen, and a

further addition of serum above that is necessary for the neutralization of the anticoagulant and the necessary prothrombin or prothrombin-antithrombin compound, will not result in any further acceleration of the clotting.

The addition of cephalin solution to the plasma containing 0.25 per cent sodium citrate, without the addition of any free calcium containing substance, also affects the rapidity of coagulation; so, for example, when one drop of 1 per cent cephalin solution was added to ten drops of plasma, a clot was formed in thirty minutes; when five drops of cephalin were added, a clot was formed in twelve minutes and so on, although in certain instances, as seen in table 12, only an imperfect clot was formed. This would tend to indicate that the cephalin is able to stimulate the clotting of the blood, even without any excess of calcium salts and in the presence of a slight quantity of anticoagulant (sodium citrate); this would be in direct opposition to the well accepted theory of the necessity of calcium salts for the process of coagulation of the blood. The only explanation that could be suggested at present would be that whether due to the presence of cephalin or not, the calcium citrate is ionized in the plasma-cephalin-water mixture and the calcium ions play their part in the process of coagulation, which is therefore accelerated; upon the addition of only a small quantity of calcium the coagulation takes place much more rapidly. The excess of soluble oxalate, the calcium salt of which is much less soluble than the citrate, will not allow this action to take place so readily.

The presence in the mixture of Ca-ions, citrate-ions and prothrombin would act, according to the mass law, in the following manner: in the presence of a large excess of the citrate ions, all the calcium will be precipitated as calcium citrate; in the presence of only a very small excess of citrate ions, the prothrombin of the plasma will then be able to combine with some of the Ca-ions and produce thrombin. This can be represented by the following formulae:

$$\text{Prothrombin excess} \leftarrow \text{Ca ions} \rightarrow \text{Citrate ions excess.}$$

That this holds true is also seen from the behavior of the oxalate in the plasma. When the coagulation of the plasma is prevented by the use of a soluble oxalate, the calcium will be precipitated out to a much greater extent, since the oxalate ions have a much greater affinity for the Ca-ions than do the citrate ions.

The data in table 13 will throw some light upon the nature of the changes that take place in old serum; while three drops of fresh serum

will clot ten drops of plasma in eighty-nine minutes and in the presence of two drops of cephalin in nine minutes, three drops of old serum will only clot the plasma in twenty-four hours, but in the presence of cephalin in ten minutes; the same relation holds true with the larger amount of old serum. This deficiency in the old serum can be corrected by the addition of Ca $(OH)_2$, showing that the change that has taken place in the serum might have had something to do with the calcium transformation in the serum. The old serum has no deleterious effect upon the fresh serum, it has even a stimulating effect:

TABLE 13

The action of old serum upon the clotting of the blood

Ten drops of plasma containing 0.25 per cent sodium citrate, horse serum 7 days old, fresh horse serum and $Ca(OH)_2$ solution.

OLD SERUM	FRESH SERUM	$Ca(OH)_2$	CEPHALIN	CLOT FORMATION
drops	*drops*	*drops*	*drops*	
3				24 hours
3			2	10 minutes
3		2		15 minutes
3		2	2	5 minutes
3	3		2	6½ minutes
5	5		2	6½ minutes
5				12 hours
5			2	8 minutes
5		2		23 minutes
		2	2	5 minutes
	3			89 minutes
	3		2	9 minutes
	5			72 minutes
	5		2	7 minutes
		2		60 minutes
		2	2	10 minutes

while three drops of fresh serum, in the presence of cephalin, produced a clot in nine minutes and five drops in seven minutes, upon the addition of three drops of old serum to these the clot was produced in six and one-half minutes. Since, according to Hurwitz, the thrombin is changed in old serum to metathrombin, it is possible that the serum has not become yet old enough for all the thrombin to change into another form.

The presence of both calcium and serum accelerate coagulation; these act in a manner similar to a large amount of serum.

The function of cephalin consists therefore in neutralizing the antithrombin of the normal blood, according to Howell's theory; the increased rapidity of coagulation in the presence of calcium salts or fresh serum is due to the fact that calcium is, as is generally accepted, an important factor in the process of the coagulation of the blood; the action of the serum consists then both in supplying calcium to the plasma-cephalin mixture and in supplying a larger amount of prothrombin.

A detailed study of clinical uses of cephalin will be published elsewhere; a large number of clinical observations on the use of cephalin has accumulated tending to show the great value of cephalin in quickly arresting hemorrhages from bone, kidney, muscle and other tissue surfaces, as well as to the bleeding wounds of hemophiliacs.

TABLE 14

The action of cephalin gauze upon the rapidity of clotting of blood plasma

Clot formation in minutes

TEMPERATURE	VACUATED	EXPOSED	CONTROL GAUZE
20°	17	25	62
37°	7	10	24

In view of the fact that cephalin in water solution rapidly deteriorates and also since it is usually necessary to have it in a sterile form, a method was worked out[1] by which surgical gauze is soaked in an ether solution of cephalin for a certain definite period of time; the ether is allowed to evaporate and the gauze impregnated with cephalin is packed in suitable containers and sterilized. It is not here the place to give the details of the methods and use of this marketable preparation; mention will only be made of the effectivity of this gauze, both kept in a vacuum and in an open container, in coagulating blood.

The same method as outlined above was used; the gauze used in this experiment was kept for about half a minute in a 5 per cent ether solution of cephalin; the gauze was sterilized in the autoclave at fifteen pounds pressure for half an hour. Three cubic centimeters of horse plasma containing 0.25 per cent sodium citrate were introduced into the glass containers, three drops of $Ca(OH)_2$, five drops of distilled water and about one-half square inch of the gauze finely cut were added to that. The data are given in table 14.

[1] Also suggested by Cecil (14).

It is thus seen that the cephalin-impregnated surgical gauze exerts the same thromboplastic action as the cephalin itself and although in this case the differences are not so striking as in the case of the latter, we can explain it by the fact that cephalin acts in solution and it takes a few minutes before the cephalin from the gauze becomes dissolved in the blood fluid.

SUMMARY

1. In testing the thromboplastic action of cephalin the method of Howell has been adopted with the following modifications:

a. Blood plasma containing 0.2 per cent to 0.25 per cent of sodium citrate was found to be well suited for these tests.

b. A standard solution of Ca $(OH)_2$ can be substituted for serum, thus eliminating one or more unknown factors.

2. The function of the serum in the plasma-serum-cephalin mixture consists in supplying the calcium necessary for neutralizing the excess of anticoagulant in the plasma and probably in supplying more prothrombin.

3. By using Ca $(OH)_2$ to neutralize the excess of anticoagulant in the plasma, an optimum concentration is found, above which the excess of calcium will delay the coagulation of the plasma.

4. There is always a maximum concentration of the cephalin in the water solution which gives the most rapid coagulation; a further increase in the concentration of the cephalin will not result in an increase in the rapidity of coagulation; by decreasing the concentration of the cephalin below this maximum a delay in the coagulation will result. This concentration seems to fall between 0.25 per cent and 0.30 per cent for the lots tested.

5. Cephalin kept in a vacuum acts much better as a thromboplastic agent than the same lot of cephalin kept in loosely stoppered containers.

6. The cephalin obtained from the brains of different animals does not differ greatly as a thromboplastic agent; the slight differences obtained were probably due to the impurities and methods of keeping the material.

7. Cephalin dissolved in water loses to some extent its thromboplastic properties, particularly old lots of cephalin kept exposed to the air.

8. Since coagulation of the blood is an enzymatic phenomenon it is much more rapid both in the presence and in the absence of cephalin at 37° than at room temperature.

9. Serum seven days old is less active than fresh serum in accelerating the clotting of the blood; this was corrected, with the method used, by the addition of a solution of Ca $(OH)_2$; fresh serum is not depreciated in its action upon the plasma-cephalin mixture by the presence of old serum but is even slightly accelerated.

10. Surgical gauze impregnated with cephalin was also tested by the above method and found to increase the rapidity of the coagulation of the plasma.

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BIBLIOGRAPHY

(1) Morawitz and Bierich: Arch. f. exper. Path. u. Pharm., 1907, lvi, 115.
(2) Whipple: Arch. Int. Med., 1912, ix, 365; Ibid., 1913, xii, 637.
(3) Howell: This Journal, 1910, xxvi, 435.
(4) Howell: This Journal, 1911, xxix, 187.
(5) Howell: This Journal, 1912, xxxi, 1.
(6) Hammarsten: Zeitschr. physiol. Chem., 1896, xxii, 333.
(7) McLean: This Journal, 1916, xli, 250.
(8) McLean: This Journal, 1917, xliii, 586.
(9) Hirschfelder: Lancet, 1915, ii, 542.
(10) Hurwitz: Amer. Journ. Med. Sci., 1917, cliv, 689.
(11) Rich: This Journal, 1917, xliii, 549.
(12) Addis: Journ. Path. Bact., 1911 xv, 247.
(13) Morawitz: Handb. d. Biochem. Arbeitsmethoden, 1911, v, 223
(14) Cecil: Journ. Amer. Med. Assoc., 1917, lxviii, 628.

THE ACTIVITIES OF DECEREBRATE AND DECEREBELLATE CHICKS

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The investigation reported in part in this paper is an attempt to throw additional light on the nature of the influence exerted by the cerebrum over the lower nervous centers in a form, the domestic fowl, in which the importance of this part of the nervous system is conspicuously less than in man. That birds are relatively little affected by removal of all that part of the cerebrum in front of the optic thalami has long been known (1). The standard picture of the decerebrate pigeon is too familiar to call for detailed repetition here. We need merely recall that locomotor activities, even those so complex as the act of flying, are preserved without demonstrable impairment while functions clearly dependent on associative memory are lost or seriously affected.

In connection with a consideration of the process of acquirement of various complex activities during the early life of the individual, it occurred to us that observations of decerebrate young birds might afford some information concerning the relative parts played by the cerebrum and the lower brain centers therein. This paper deals with the behavior of the forms studied. Subsequent communications will report the results of anatomical studies and observations on vision.

Material. Our observations were made upon young chicks. Of these the first lot, seven in number, were hatched under a hen, February 1 to 3, 1917. The second lot of twenty-nine were incubator hatched, March 1, 1917, as were also the third lot of six, March 31, 1917. All the chicks were of the white leghorn breed. We had no difficulty in maintaining them in good health. Of the entire series only two, both from the first lot, developed any characteristic ailments. These became "droopy" and died on the sixth and seventh days respectively. A feature of the chick that makes it a favorable object for such a study as this is the presence at hatching of a considerable residue of yolk which nourishes the bird during the first two days.

Operative Procedure. The operations performed were as follows:

1. "Standard" decerebration. In this operation we attempted to repeat, so far as possible, the usual procedure for preparing decerebrate pigeons for class demonstrations. A longitudinal skin incision was made along the median line of the skull; then with stout scissors skull and dura were cut through transversely just anterior to the fronto-parietal suture. This cut was about 5 mm. long, approximately half on each side of the midline. From the ends of this first cut other cuts were made forward for about 3 mm. The flap of bone resulting from these cuts was lifted and the tip of a glass tube connecting with an aspirating pump was inserted. We found a glass tube as large as could be passed readily through the opening of the skull (outside diameter of tip 4 mm.) more satisfactory than a smaller tube. By means of this tube the brain substance and blood were sucked out of the cranial cavity. By watching through the opening and taking only that brain substance which came freely, the desired amount was obtained in every case. The hemorrhage was not severe and no fatalities occurred from this operation. Since the operation, to and including removal of the hemispheres, occupied only about five seconds, anesthesia was not employed.

2. "Deep" decerebration. In this procedure we attempted to remove, in addition to the mass of brain substance taken in the "standard" operation, a small additional amount from the thalamic region. After the hemispheres had been removed in the manner described above, the suction tube was thrust into contact with the brain substance still visible on the floor of the cranial cavity and a small mass withdrawn. As variations on this method we tried cutting away a small slice with fine curved scissors or with a sharp bent knife.

3. "Shallow" decerebration. In birds the pallium is very thin and according to certain investigators (2) has less importance in determining the nervous activities of these creatures than the underlying corpora striata. We attempted to remove the pallium and leave intact the corpora striata by making the opening through the skull with great care so as to avoid injuring parts beneath, using a much smaller suction tube (2 mm.) and keeping the tube always parallel to the roof of the skull so that superficial structures might be withdrawn without disturbing deeper ones.

4. Unilateral decerebration. As the name implies, this consisted in the removal of only one or the other hemisphere. Except in the case of chicks just hatched, ether anesthesia was employed during this

operation. We obtained unilateral "standard" decerebration and also, in a single case, unilateral "deep" decerebration. The procedure was similar to that described above except that the skin and skull incisions were confined to the side of the midline from which cerebral tissue was to be taken.

5. Ablation of the cerebellum. We were successful in one of two trials in removing a considerable portion of the cerebellum of a new hatched chick. This was accomplished by suction through an opening made with a 3 mm. drill through the parietal bone lateral to the midline. The operation was performed without anesthesia. There was more hemorrhage than in standard decerebration but not enough to indicate that the effects observed were due to loss of blood.

Care of the chicks. Since decerebrate birds do not feed themselves successfully nor drink spontaneously a technique for giving the chicks food and drink had to be adopted. After consultation with professional poultry breeders and considerable experimentation we settled upon the following ration. Dry bread was pulverized in a mortar and then thoroughly mixed with hard boiled egg, shell included, in the proportion of two parts bread to one of egg. To this a small amount of charcoal and some finely chopped alfalfa hay were added. This mixture was dried in the sun. When about to be fed a sufficient portion of it was moistened with water to permit it to be formed into small pellets. The chicks were fed by holding the beak open and inserting a pellet into the throat whence it was swallowed as soon as the beak was released. In addition to this food mixture a commercial chick food consisting of mixed grains and seeds chopped fine, was administered, together with a small amount of "grit." The average feeding consisted of 1.5 grams of the food mixture and 1 to 4 grams of grain and grit. The chicks were fed twice daily. In connection with each feeding as much water was given as would be taken without appearance of distress. Usually additional water was given at intervals during the day. A fixed rule was adopted that the crop must be empty at least once daily. If it was found to contain food at any feeding time only water was administered. The amount of food given was controlled by weighing each chick before and after each feeding.

Behavior of young normal chicks. Careful studies of the activities of young chicks have been made by various observers (3), (4), (5), (6). For our purposes it is convenient to attempt to classify the activities described by these investigators into fairly related groups. Four such suggest themselves. *a. Locomotor activities.* By these we mean

walking, running, flapping of wings, jumping up into a support or down from one, balancing on a narrow perch, craning the neck downward from a perch and other movements obviously allied to these. *b. Self cleaning activities*, including preening self, scratching self, wiping bill. *c. Feeding activities.* Pecking at moving and stationary objects, seizing and swallowing food, scratching in litter, drinking. *d. Miscellaneous activities apparently of a higher order:* tendency to run to moving objects, including other chicks; tendency to seek solitude when in possession of a large tidbit; tendency to attempt to seize such a tidbit from another chick; the "fear reaction" (see below); the manifestation of "wildness" when approached; acts apparently based on memory such as the deliberate return to the brooder after escaping from it. By the "fear reaction" we mean the very characteristic behavior of young chicks, described by Thorndike (3) and others, in response to strange sounds or objects. We found shrill "trilling" admirably adapted for eliciting this reaction. In our experience this sound was much more effective than the "mew" used by Thorndike. When the stimulus is given the chicks dart rapidly in various directions and then become immobile, remaining so for some time, a minute or more in certain cases. The attitude during immobility is often very striking Usually the chick crouches and holds the head in the air, with neck slightly bent so that one eye is directed toward the sky. Sometimes immobility overtakes the chick in an unusual situation. On one occasion we observed a chick immobile in the act of stepping over the rather high threshold of the warm chamber. He remained motionless with one leg outside and the other inside for fully thirty seconds.

The time of appearance of the various activities as observed by ourselves and reported by other investigators is substantially as follows: The activities of group *a*, locomotor actions, begin to appear very soon after hatching. Most chicks walk and flap their wings on the first day and run, jump, and balance well by the second or third. One gains the impression from watching them that the manifestion of activities of this class waits on the attainment of sufficient muscular strength rather than on any particular stage of brain development. In other words, one is tempted to conclude that the nervous mechanism of locomotion is adequate at the time of hatching but that the muscles do not attain adequacy for some hours thereafter (Thorndike, p. 286).

In group *b* the "preening" reaction is first to appear, being shown by chicks only a few hours old. Scratching of self and wiping bill are occasionally seen on the first day and typically on the second or third.

The activities of group *c* seem to appear in fairly definite order. Most chicks follow and peck at small moving objects, such as the metal tip of a pencil, within twelve hours of hatching; often before walking itself is well performed. Pecking at stationary objects, grains or specks, appears almost as soon in some, a day later in others. A few precocious chicks seize and swallow grains with considerable accuracy on the first day. Most do not do so till the second day and Breed (5) reports improvement in accuracy of seizing up to the third day and attainment of ultimate accuracy by the eleventh day. We did not make quantitative observations on accuracy of seizing but noted that most of our chicks were obtaining enough food on the second day to distend their crops.

We did not observe definite scratching in litter till the third day, although we noted in two instances on the second day what appeared like feeble beginnings of the action. Drinking is learned by most chicks as early as the second day, and chicks will make characteristic movements, lifting head and swallowing, within twelve hours of hatching, if the bill is dipped into water.

Some of the activities of group *d* appear early; others are not seen till a considerable time after hatching. We observed a pronounced "fear reaction" on the second day, and Morgan (6) cites an observer to the effect that a type of fear reaction may occur in certain species of birds even before hatching. The tendency to run toward moving objects is clearly manifest by the third day. The characteristic displays of greediness, snatching at food in other chicks' mouths and attempting to prevent this, we observed first on the twelfth day. "Wildness" is said by Thorndike (loc. cit., p. 288) to develop during the first month, with possible beginnings as early as the tenth day. We have seen it well developed in chicks two weeks old.

The reaction which we mentioned above as suggesting memory, namely the deliberate return (by jumping over a wooden wall one foot high) to the brooder whence escape had been made, we noted first on the sixth day. Thereafter the chicks jumped out and in with great freedom.

Behavior of "standard" decerebrates. For purposes of description these will be placed in series according to age after hatching at which decerebration was performed. The first series includes four chicks decerebrated within an hour or so after hatching, in every case before becoming dry. In the second series are four chicks decerebrated between the third and eighth days. The third series consists of three

chicks decerebrated between the tenth and the twenty-sixth days. The first series is in general the most interesting since in its members the cerebral hemispheres may reasonably be supposed not to have commenced functioning actively at the time they were removed. The entire nervous development of these chicks might be said to have occurred, then, independently of cerebral influences.

In general the locomotor activities (group *a*, p. 398) appear to develop in these early decerebrates as rapidly as in normals. The "shock" of operation seems to be very transitory. In two to four hours after it is performed the chicks are in general appearance on a par with normals of the same age. Three of those observed by us walked well before the end of the first day; the fourth stood unsteadily on the first day and walked well on the second. They jumped, flapped their wings, and perched about as early as normals. One jumped out of a chalk box on the third day, another on the fifth, and another on the seventh. We had the impression in the case of those that were slow about performing this feat that absence of stimulation rather than lack of ability was responsible. The decerebrates placed within the chalk box tended at once to show the drowsiness so characteristic of decerebrate birds. This drowsiness is not so persistent in young decerebrates as in older ones and we were always rewarded at one time or another with attempts to escape from the box. Some of the decerebrates were definitely less skilful in this feat than any normals of the same age but the difference did not seem to us sufficiently marked to be undoubtedly significant. The self-cleaning activities (group *b*, p. 399) are also as prompt in appearance in these decerebrates as in normals. They preened themselves and wiped their bills on the first day and scratched themselves on the second.

In the feeding activities (group *c*, p. 399) the first striking differences begin to appear. Pecking at both moving and stationary objects is established by the second day but the actual seizing and swallowing of food, activities which, in normal chicks, follow closely upon the commencement of pecking, fail to progress beyond what might be called the accidental stage. If one of these chicks is placed on a table on which grains are scattered it pecks frequently although rather aimlessly. At rare intervals a grain is taken into the beak. A normal chick, after the third day, frequently mouths grains that are seized and works them back into a position whence they can be swallowed. We have not seen standard decerebrates do this. Apparently any grains that are swallowed are such as in the act of seizing happen to strike far enough back in the mouth to evoke the swallowing reflex.

Scratching on the floor of the brooder or in litter was first seen in a chick of this group on the sixteenth day. In normal chicks as previously noted, scratching is well established by the third day. These decerebrates appear to scratch in much the same manner as do normals, although they show even less discrimination than the latter in the selection of spots where the act might have some degree of utility. They are by no means so persistent in scratching as are normals.

A striking fact with regard to these decerebrates is the complete disappearance in them of spontaneous drinking. If their bills are dipped in water they make appropriate drinking movements, or if they peck accidentally in the drinking vessel they do likewise, but we noted no suggestion of deliberate attempt to obtain water nor did we observe that characteristic "scooping" movement described by Breed (loc. cit., p. 8) as preliminary to and included in the act of drinking. They were often longer without water than the normals but in spite of their presumably greater thirst the reaction did not appear.

Of the miscellaneous reactions (group *d*, p. 399) the most conspicuous in these chicks, decerebrate from hatching, is the tendency to run toward moving objects. This is very striking particularly during the first fortnight. Movement of a bright object in their field of vision will almost always cause them to approach quickly. Whenever any chick runs for any reason the decerebrates run with him. In connection with the "greed" reaction the decerebrates run in close company with the normals that are engaged in pursuing the possessor of a tidbit but we have not seen any of them attempt to snatch the latter, as normals continually do.

The "fear reaction" is either wholly absent from these decerebrates or only its initial manifestation is present. Usually the decerebrates pay no attention to the trilling. We have seen a few instances of apparent reaction to it but only in the form of rapid darting for a few steps. Instead of the subsequent immobilization, seen in normals, these resume promptly their former activities. "Wildness" is wholly absent. These chicks offer no objections to handling or to the approach of the hands. Acts clearly dependent on memory were not observed to occur.

Series 2. The second series of standard decerebrates, those in which the operation was performed between the third and eighth days, showed some interesting contrasts in comparison with the chicks decerebrated

at hatching. These chicks before decerebration fed themselves, drank, scratched in litter, showed the "fear reaction" and behaved generally in a manner to indicate that the normal activities were well established.

Our special interest in this series was to observe whether any of the activities which fail to appear or which are slow in appearing in chicks decerebrated from hatching would show themselves, or appear sooner in these, decerebrated after the activities were well developed. Our observation was that the locomotor and self cleaning activities, which develop normally in the early decerebrates, continue unimpaired in these. We noted one feature of locomotion which seems worthy of record. In all the chicks decerebrated after the second day there was a period, starting the day after decerebration and continuing for several days thereafter, in which there was a pronounced tendency to run in straight lines. The chicks would start and run till their course brought them to the wall of the brooder. Thence they would follow along the wall, slowing down abruptly at the corners, turning them skilfully, and proceeding. Certain chicks kept the wall always at their right, others kept it always at their left. This tendency was rendered striking by the circumstance that the inclined approach to the warm chamber of the brooder was so placed that those keeping the wall to the right would always run up it and into the warm chamber. This would be encircled, then the chick would emerge, swing sharply to the right, jump off the edge of the incline and proceed. Those that ran with the wall at the left were never seen to enter the warm chamber in this manner. They would approach the incline from the side, pass along its foot, and continue around the edge of the brooder. This running tendency disappeared after a few days and we are inclined to attribute it to some irritation from the seat of injury.

In connection with this running reaction we observed a typical manifestation of the decerebrate condition. The brooder was so placed that at a certain hour a bright strip of sunlight about 2 cm. wide lay along the floor in the path of chicks running along the wall. Two different chicks were observed repeatedly as they approached this bar of sunlight to jump over it as over a physical obstruction; none of the normals behaved similarly.

The feeding activities in these chicks corresponded in general with those of the early decerebrates with one exception. Whereas none of the latter began to scratch on the floor before the sixteenth day, all those decerebrated between the third and eighth day resumed scratch-

ing within eight days. All the decerebrates of this series were scratching at least three days younger than the youngest early decerebrate to begin the act.

The miscellaneous activities of these chicks were the same, so far as our observation extended, as those recorded above for the early decerebrates.

Series 3. The chicks decerebrated after the eighth day were grouped separately from those just discussed because of their different behavior in connection with the feeding activities. Instead of preserving the reaction of scratching unimpaired or resuming it after a short delay, as we rather expected from our observations on the other groups, we were surprised to note that the scratching activity disappeared completely and was not resumed. One of these chicks, decerebrated on the tenth day, lived forty-two days thereafter, showing during that time most of the activities common to decerebrate chicks, but without scratching, at least during the time of observation which included most of the daylight hours. The pecking activity seemed also to be less manifest in the chicks decerebrated later than in those decerebrated young. The chick mentioned above, decerebrated on the tenth day, was not seen to peck at all for six days and after that only at occasional intervals during a month. A chick, decerebrated on the twenty-sixth day, had not been seen to peck up to the time it was killed on the thirty-third day.

In general all the standard decerebrates were more active during the first month of life than later. This appeared to hold irrespective of the age at decerebration. Those decerebrated early showed a long period of activity, while those decerebrated late lapsed quickly into a sluggish state. The appearance of all these decerebrates at the end of a month of life corresponded closely with the usual description of the adult decerebrate pigeon. Occasional intervals of activity, probably associated with hunger, alternated with periods of standing quietly in one spot, apparently sleeping. All these chicks showed the characteristic drooping of the feathers described for decerebrate birds, developing gradually and finally becoming very marked.

"Shallow" decerebrates. In this category were four chicks. Two of these were prepared with great care to keep the injury superficial. In the other two an attempt was made to obtain a condition intermediate between the standard and the shallow decerebration. Of the two designedly "shallow" decerebrates, one was operated on at hatching, the other on the eighteenth day. One of the two intermediates

was prepared at hatching, the other on the fifth day. All are grouped together because in the main their reactions were similar. All fed themselves successfully, drank spontaneously, scratched actively and in general deported themselves like normals. Certain minor features of difference were noted. On the whole these chicks appeared to have less initiative than normals and to be less "wild." One or two of them showed remarkable subservience to external stimuli. For example, repeated tapping on a hard surface sufficed to attract one to the point tapped. By the use of this stimulus this chick could be led all over the laboratory or caused to jump up into a chair and thence to a table and down again. It reacted in this manner on a demonstration table before an audience, a situation in which normal chicks show signs only of fright or bewilderment.

One of the "intermediates" was interesting in that while most of his activities were similar to those of the "shallow" decerebrates, his behavior in scratching resembled that of "standard" decerebrates. This chick was prepared at hatching. He was first seen to scratch on the floor on the twentieth day thereafter. None of these chicks showed signs of operative "shock" in any noteworthy degree. Those prepared some days after hatching resumed their usual activities within three or four hours after operation. Those prepared at hatching appeared to develop as rapidly as normals of the same age.

"Deep" decerebrates. We attempted on six chicks the operation described above as "deep decerebration," namely the mutilation of some portion of the thalamus, in addition to complete removal of the portions anterior thereto.[1] These chicks ranged in age between five and fourteen days. We did not try this operation on any new hatched chicks. Three of the six chicks in this series died within twenty-four hours. The others lived three, five and six days, respectively. Those that died early showed few significant features. A tendency to rigidity (decerebrate?) was noted in two of them immediately following operation. One of them struggled wildly and presented an appearance which would, in a normal chick, suggest suffering—eyes staring and bill open. Another stood and shook its head when disturbed; it remained quietly in one spot except when stimulated.

All the three chicks that lived more than one day showed the swallowing reflex and all stood and walked about. In each case the walking was unsteady and presented an appearance of effort. There was manifest

[1] From one chick, no. 15, only the right cerebral hemisphere was removed. This chick lived only six days.

difficulty in maintaining equilibrium together with apparent muscular weakness. The visual reflexes seemed to be present. One chick, No. 22, that lived five days, was seen to preen itself on the first day before it had attempted to stand. On the fourth day it attempted repeatedly, while standing, to preen the feathers on the back near the tail. On four trials it toppled over backward, recovering itself after violent struggling. At a fifth trial it succeeded in making the preening movements without falling.

Chick 15, from which only one cerebral hemisphere had been removed, behaved in the same general manner as the other deep decerebrates. It showed no activities that were not shown by other deep decerebrates from which both hemispheres had been removed. It was observed to hold its tail toward the uninjured (left) side. Its compensatory tail movements were noted to be slight in comparison with those of normal chicks or of half-decerebrates in which the thalamus was intact.

All these deep decerebrates were much more active, and to judge from appearance, in much better condition when in a warm environment than when placed in a cool spot. The difference was obvious. We did not attempt to determine whether or not there was an actual lowering of body temperature in the cool surroundings although the difference in behavior suggested that such a lowering might occur (8).

Unilateral decerebration. Beside the half decerebration described above, we removed one cerebral hemisphere from each of four chicks, two at hatching and two at two weeks old. In one of each age the left hemisphere was removed, in the other the right. Our aim in these experiments was primarily to observe the effects of "shock" as distinct from those of removal of nerve substance. We were and are of the opinion that surgically the removal of one hemisphere constitutes as pronounced a basis of "shock" as the complete ablation of the cerebrum. The observations of interest on these chicks had to do, for the most part, with the entire absence of disturbances attributable to "shock." None of them showed more than a transitory interruption of function. Except on close examination they were indistinguishable from normals of corresponding age. The most obvious difference between them and normals was in the matter of vision. All the half decerebrates retained normal vision on the side of injury and showed visual reflexes, but not psychic vision, on the side opposite the injury.[2]

[2] A detailed study of vision in these chicks will be presented in a separate communication.

Ablation of the cerebellum. The single chick in which ablation of the cerebellum was successfully accomplished lived six days thereafter. The following were the chief points noted in respect to behavior: The chick made no attempt to stand; except when held it lay on side or back. It was very active, struggling almost continuously. Its most violent movement consisted in kicking out with both feet together. There was also much flapping of wings. When held in the hand with feet curled up to the body it would remain quiet. Any attempt to put it down led to a renewal of the violent kicking so that it had to be kept closely wrapped to prevent self injury. There was loud and persistent peeping much of the time. The head was thrown back and forth repeatedly. There seemed to be no pronounced loss of strength during the first five days; on the sixth day weakness became pronounced. The movements of the legs appeared to be wholly incoördinate, although the chick was able to make coördinated movements of some parts. Thus when held in the hand, the situation in which it appeared to be most comfortable, the chick made directed movements of head and neck. When the bill was dipped in water a typical drinking reaction occurred. This was seen on the first and second days. It was successful only when not too extensive. If the head happened to be raised too far there succeeded to the drinking movement violent backward and forward motions of the head. On the fifth day the chick preened itself on the wing, pecked at a moving pencil point and made what appeared to be pecking movements at grain placed within easy reach. All these activities occurred while the chick was being held in the hand. So far as we could judge, hearing was normal as well as sight. Careful tests for compensatory movements were made for us by Professor Weymouth. None could be demonstrated although their presence could not be absolutely excluded. In comparison with the conspicuous manifestations of equilibrating power in standard decerebrates, compensation in this chick was certainly negligible.

DISCUSSION

Although comprehensive analysis of the observations described in this paper must wait for the completion of the morphological studies now in progress on the operated brains, there are certain points which seem sufficiently clear to justify comment.

Our observations seem to us to demonstrate that in chicks locomotor and self cleaning activities develop and are mediated in complete

independence of the cerebrum. The first definite suggestion of cerebral influence is seen in connection with feeding activities. The distinction suggested by Breed (5) between striking at grains and actually seizing and swallowing them, appears from our work to have a definite anatomical basis. Thus our "standard" decerebrates developed the pecking reaction as far as "striking" almost as early as normals. The further progress of the reaction to the point of successful self feeding, which in normals occurs within twenty-four to forty-eight hours after the initiation of "striking," fails in the decerebrates completely. The suggestion is that the act of "striking" is in a category with locomotion and self-cleaning, whereas "seizing" and "swallowing" belong in a different category and one which is dependent on the cerebrum.

More complex is the analysis of the act of scratching in litter or on the ground. In normal chicks this is established by the third day and continues thereafter throughout the normal life of the individual. In none of our "standard" decerebrates did this activity appear so promptly although it did appear ultimately in all in which decerebration was performed within eight days after hatching. We have here a suggestion of an activity that is not necessarily dependent on the cerebrum but under normal circumstances develops in relationship with it. Our observations suggest, further, that the ability of the lower parts of the nervous system to establish this reaction, independently of the cerebrum, is quite limited. The fact that none of our chicks, decerebrated after the tenth day, reacquired it, indicates a nervous plasticity during the first days that does not persist thereafter. Confirmatory of this idea is our observation of the increasing general sluggishness after the first month. In fact studies of the learning process as a whole in chicks suggest that the period of plasticity is confined to the first few days (Breed, loc. cit.). That young decerebrate kittens are similarly more responsive than adult cats has recently been shown by Weed (7).

In sharp contrast with these feeding activities is the associated act of spontaneous drinking which, as we have noted, disappears at once from a bird decerebrated after it is established and fails completely to develop in one decerebrated at hatching. Functionally this act ranks in immediate importance above pecking or even above seizing and swallowing, and is undoubtedly far more important than scratching in litter, yet it is perhaps the least deep-seated of all the feeding activities. Any attempt to account for the failure of representation of so funda-

mental an act in the underlying portions of the nervous system must be purely speculative. The suggestion may be permitted, however, that drinking is a necessary activity only in land forms. Feeding movements, on the other hand, occur as well in aquatic animals. If we assume that the establishment of function within the sub-cerebral nervous structures took place during the period when life was predominantly marine and when, therefore, deliberate drinking was unnecessary, we can see why the nervous mechanism of drinking might not have arisen within these lower nervous regions, but in a region, the cerebrum, of later development.

We do not feel disposed to enter upon any discussion of the influence of decerebration upon the complex activities listed by us under group *d*. We have described modifications of behavior which seem to us significant but offer them rather as contributions to knowledge than as the basis of definite conclusions.

The comparison of "shallow," "standard" and "deep" decerebration confirms the conception previously established for adult birds, that the impairment of function is more profound the more extensive the injury. There is still room for question, however, whether the marked effects of "deep" decerebration are due to interruption of important nerve pathways or whether they are secondary in character, resulting from disturbance of the temperature-regulating or some other "vital" mechanism.

The negligible impairment of function following unilateral decerebration, when not involving injury to the thalamus, demonstrates first, that the effects of our other procedures were not due to operative shock; and second, that one cerebral hemisphere suffices in birds for the usual range of their activities.

Our observations on a decerebellate chick are chiefly confirmatory of current views. Complete incoördination of the muscles concerned with locomotion, associated with marked and almost continuous struggling, was the most striking feature. There was a definite but limited control over head movements. Whether this was due to failure to ablate the entire cerebellum or whether there is such a limited control independently of the cerebellum can be decided only on the basis of further work.

SUMMARY

1. Three types of decerebration were performed on newly-hatched or very young chicks. These were "standard" decerebration, repetition of the usual operation as done on pigeons; "shallow" decerebration, removal of the pallium with avoidance, so far as possible, of injury to the corpora striata; "deep" decerebration, injury to the thalamus in addition to removal of the structures anterior. Unilateral decerebration of "standard" and of "deep" type was also performed.

2. The behavior of young normal chicks is considered in terms of four groups of activities: *a*, locomotor; *b*, self-cleaning; *c*, feeding; *d*, miscellaneous activities of a higher order. The age at which these appear is noted.

3. Chicks, decerebrated according to "standard" procedure immediately after hatching, develop locomotor and self-cleaning activities as early and substantially as efficiently as normals. They begin to peck about as early as normals but fail to progress beyond the act of pecking to successful seizing of food. Scratching in litter develops very slowly. Spontaneous drinking fails to appear. There is pronounced tendency to run toward moving objects. "Wildness" and fear are absent.

4. Chicks decerebrated between the third and eighth days, after the normal reactions are well established, revert to the condition of chicks decerebrated immediately after hatching. These chicks resume the activity of scratching in litter more promptly than it is developed in chicks that had not scratched at the time of decerebration. These chicks show a marked tendency for a few days to run in straight lines.

5. Chicks decerebrated after the eighth day show activities similar to those of other decerebrates except in the case of such as have to do with feeding. In those chicks there is markedly less pecking than in the other decerebrates and scratching fails completely to reappear.

6. Chicks in which the operation is confined to the ablation of the pallium, with a minimum of injury to the corpora striata, the so-called "shallow" decerebration, show only minor differences as compared with normals. There seems to be a more pronounced subservience to stimuli and less "wildness."

7. Chicks in which the operation includes injury to the thalamus, "deep" decerebration, appear weaker than the others and to have less secure equilibrium. The walking is unsteady and the act of preening is accomplished with difficulty. There is obvious impairment of

function when in a cool environment, suggesting a lowering of body temperature.

8. Unilateral decerebration has no demonstrable effect upon chicks except the loss of psychic vision on the side opposite the injury, with retention of the visual reflexes.

9. Ablation of the cerebellum brings about a condition of complete incoördination so far as locomotor movements are concerned, although there seems to be a limited power of coördination of the movements of the head. Compensatory movements are not readily demonstrable. There is much struggling and loud peeping.

10. The conclusion is drawn that the cerebrum has no necessary concern in the development and mediation of locomotor and self-cleaning activities in chicks. The successful accomplishment of feeding depends on the coöperation of the cerebrum, and the simpler phases of the act, pecking and scratching, are normally developed through the coöperation of the cerebrum, although if this is removed early enough in the life of the chick both may develop independently of it. A superior plasticity in early life is thus indicated.

11. The suggestion is offered that the complete disappearance of spontaneous drinking which follows decerebration may signify that this act, unnecessary in marine animals, may have developed comparatively late in evolutionary history, after the underlying parts of the nervous system were established in function, and concurrently with the development of the cerebrum.

BIBLIOGRAPHY

(1) FLEURENS: Recherches experimentales sur les proprietes et les fonctions du systeme nerveux dans les animaux vertebres, 1824.
(2) BAGLIONI: Handb. d. vergleich. Physiol. (Winterstein), 1913, iv, 426.
(3) THORNDIKE: Psych. Rev., ~~1899~~ 1899, vi, 282.
(4) MORGAN: Introduction to comparative psychology, London, 1903.
(5) BREED: Psychological monographs, 1911–12, no. 1.
(6) MORGAN: Habit and instinct, New York, 1896, 31.
(7) WEED: This Journal, 1917, xliii, 134.
(8) ROGERS: This Journal, 1918, xlv, 553.

THE EFFECT ON BODY TEMPERATURE INDUCED BY THERMAL STIMULATION OF THE HEAT CENTER IN THE BRAIN OF THE CAT[1]

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Aronsohn and Sachs (1) in 1885 first performed the now well known heat puncture. They punctured the corpus striatum of the rabbit producing an increase of body temperature which they attributed to increased heat production from stimulation of the corpus striatum.

Hale White (2) in 1890 localized the temperature center in the corpus striatum and optic thalamus.

In 1912, Barbour (3) found that thermal stimulation of this center in the corpus striatum in rabbits produced changes in the rectal temperature. Heating the center lowered body temperature and cooling it raised the temperature.

The experiments of this paper were performed with the purpose of throwing light on two points:

1. To ascertain whether the mechanism discovered by Barbour (3) in the rabbit is peculiar to that animal; and

2. To determine the external landmarks of the heat center in the brain of the cat, an animal which is more suitable for certain experiments than the rabbit.

METHOD

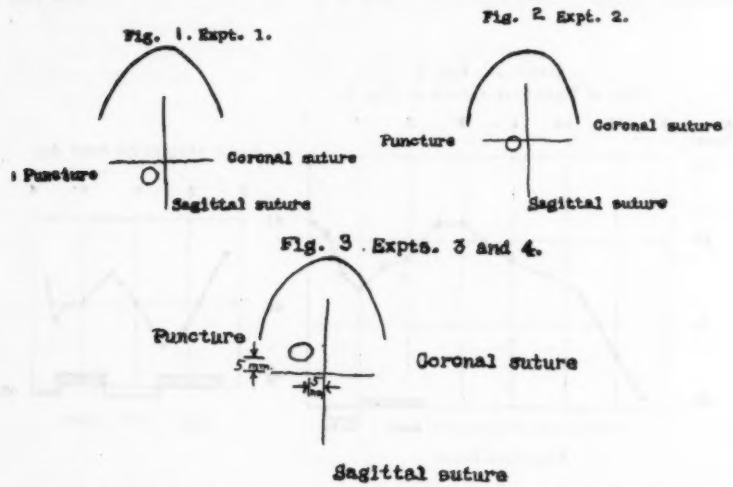
The method used was essentially that used by Barbour (3) on rabbits. Aseptic operations were performed under ether anaesthesia. The brain was punctured with a double metal tube which carried a stream of water of the desired temperature. The tube was introduced and held in position by a metal cylinder screwed into the skull. The temperature of the water running into the tube was about 10°C. for cold and about 50°C. for hot. The body temperature was measured per rectum.

[1] The expense of this research was defrayed by the Loomis Medical Research Fund.

It should be emphasized that heating and cooling of the center was always carried out after complete recovery from anaesthesia. The failure of Sachs and Green (4) to demonstrate the heat center in cats may have been due to the use of an anaesthetic.

In each experiment time was allowed for the puncture fever to assert itself before applying thermal stimulation to the brain.

In experiments 1 and 2 no constant effects on the body temperature were obtained by heating and cooling the brain (see figs. 4 and 5). It was concluded therefore that the heat center had not been punctured and that the site of puncture used in these two experiments was not the correct one (figs. 1, 2).

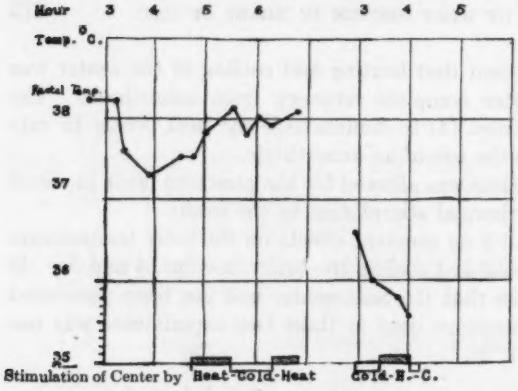


Diagrams showing site of brain puncture with reference to external landmarks. Figure 3 shows the correct external landmarks for puncture of the "heat center."

In experiments 3 and 4 constant results were obtained. Heating the brain caused a distinct fall in body temperature and cooling caused a distinct rise (see figs. 6 and 7). It was therefore concluded that in these two experiments the puncture reached the heat center. The punctures in these two cases were made 5 mm. lateral to the sagittal suture and an equal distance anterior to the coronal suture (fig. 3). A gross brain section made of the cat used in experiment 4 showed the puncture to penetrate the brain 1 mm. in front of the anterior border of the corpus striatum.

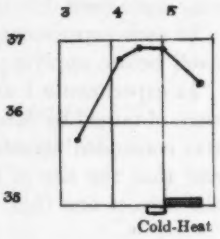
Expt. 1. Fig. 4

Site of Puncture shown in Fig. 1



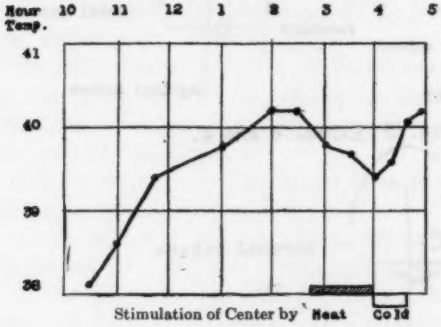
Expt. 2. Fig. 5

Site of Puncture shown in Fig. 2.

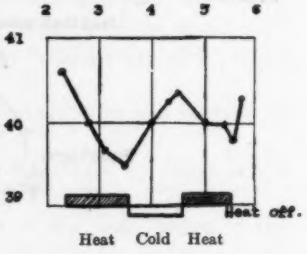


Expt. 3. Fig. 6

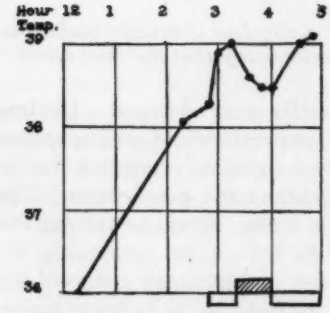
Site of Puncture shown in Fig. 3.

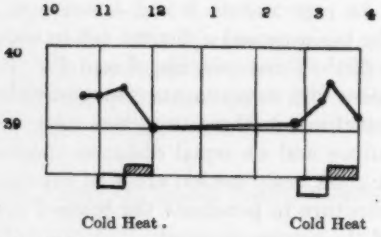


Same animal on next day



Puncture fever





Puncture fever. Expt. 4. Fig. 7

Site of Puncture shown in Fig. 3

CONCLUSIONS

1. The "heat center" in the cat is located by the same external landmarks as in the rabbit, i.e., 5 mm. lateral to the sagittal suture and the same distance anterior to the coronal suture.

2. In the cat as in the rabbit, heating the heat center induces a lowering and cooling induces a rise in the body temperature.

3. Heating and cooling of the brain at other sites does not produce a similar constant effect.

BIBLIOGRAPHY

(1) Aronsohn and Sachs: Arch. f. d. gesammt. Physiol., 1885, xxxvii, 232.
(2) White: Journ. Physiol., 1890, xi, 1.
(3) Barbour: Arch. f. Exper. Path. u. Pharm., 1912, lxx, 1.
(4) Sachs and Green: This Journal, 1917, xlii, 603.

THE EFFECT ON THE VOLUME OF THE HIND LIMB INDUCED BY HEATING AND COOLING THE CORPUS STRIATUM OF THE RABBIT

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Barbour (1) in 1912 found that thermal stimulation of the heat center in the corpus striatum in rabbits produced changes in the rectal temperature. Heating the center lowered body temperature and cooling it raised the temperature. Barbour pointed out the usefulness of this mechanism by which it appears that fever would automatically lower body temperature, and vice versa, an abnormally low temperature would tend to be raised.

In 1914 Barbour and Prince (2) showed that these changes in temperature were due in part to changes in heat production, as they were able to demonstrate that thermal stimulation of the center produced changes in the carbon dioxide output, the oxygen consumption and the respiratory volume.

Barbour had previously (1) shown that the changes in temperature were associated also with changes in heat dissipation, for heating the center caused dilatation and cooling it caused constriction of the vessels of the ear.

The experiments of this paper were devised to ascertain:

1. Whether the "heat center" in the corpus striatum controls also a vasomotor mechanism in the hind limb.

2. Whether such a mechanism involves control mainly of the vessels in the skin.

METHOD

Rabbits were used. The corpus striatum was punctured according to the method described by Barbour (1) A double metal tube which carried a stream of water of the desired temperature was introduced and held in position by a metal cylinder screwed into the skull. The corpus striatum was reached by pushing the tube through an opening

in the skull trephined at a point about 5 mm. from the sagittal suture and an equal distance in front of the coronal suture. The temperature of the water running into the tube was about 10°C. for cold and about 50°C. for hot.

In some experiments aseptic operations were performed under ether anaesthesia and the animals were allowed to come out from the ether

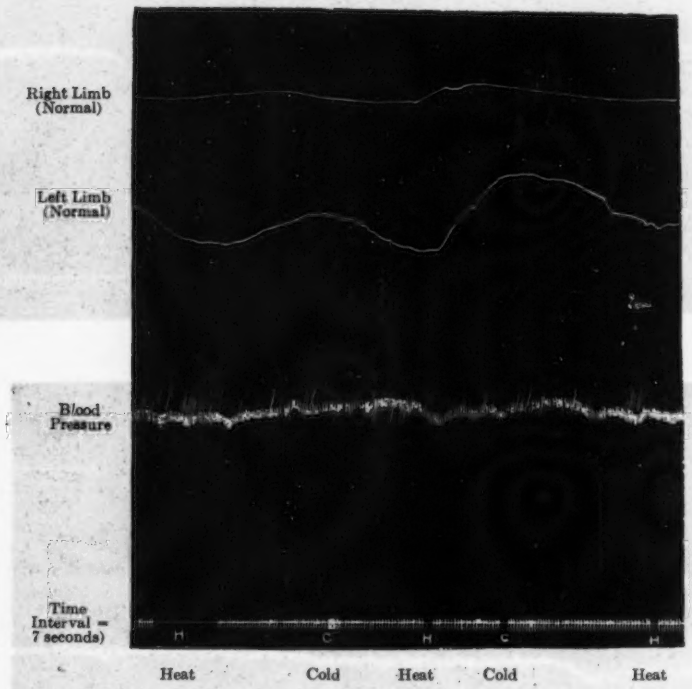

Fig. 1

to be used on the next day. When the animal was to be used only on the current day, paraldehyde was used (1.7 cc. per kilo body weight) and absolute asepsis was not considered necessary because the experiment did not last long enough for infection at the site of operation to become a factor influencing the results.

In order that changes in the temperature of the environment should not disturb the experiment, the animals were kept under conditions

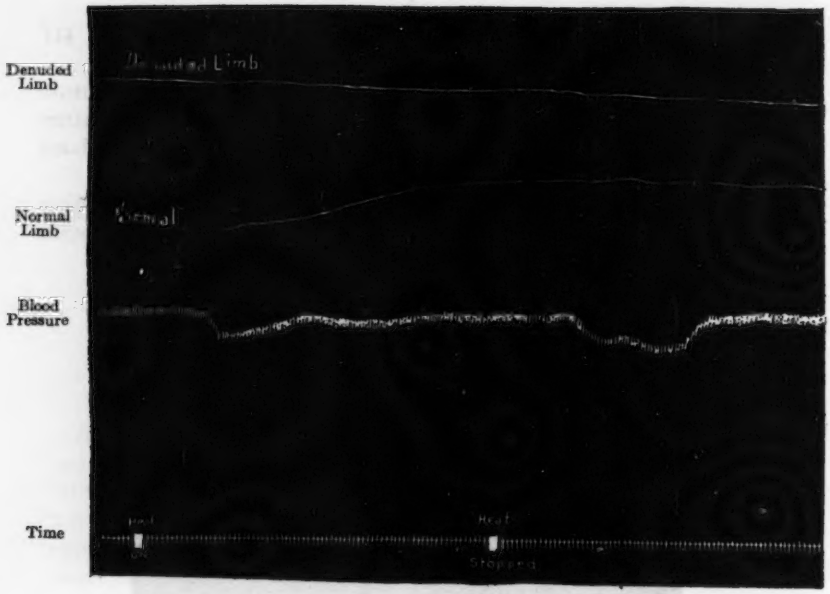
Denuded Limb
Normal Limb
Blood Pressure
Time

Fig. 2

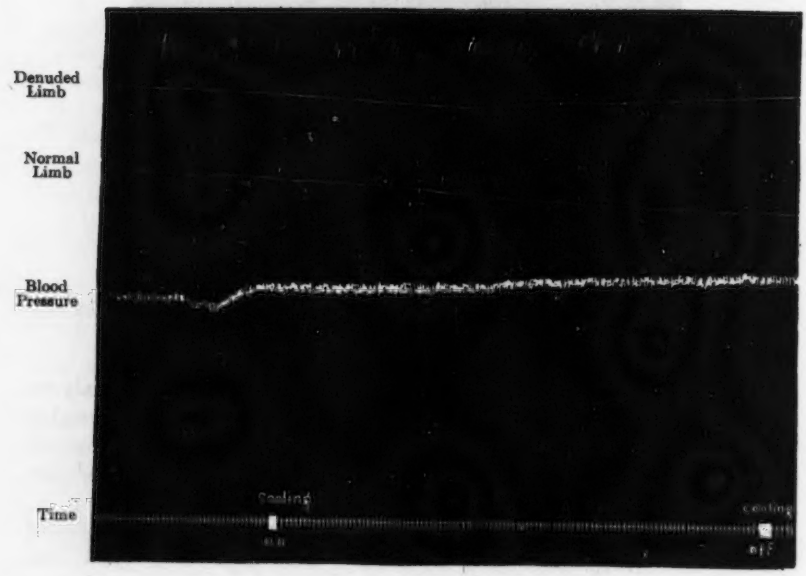
Denuded Limb
Normal Limb
Blood Pressure
Time
Cooling
Cooling

Fig. 3

conducive to a constant body temperature by being placed above a warm pan, but separated from it by a wooden stage. Blood pressure records were kept during all the experiments as a control of the volume changes in the limb.

In order to determine whether the changes in the volume of the limb were mainly effected by the skin vessels, in some experiments the skin was carefully removed from one leg while the other leg was left intact, and synchronous records were obtained from both legs. In removing the skin, special care was taken not to injure the subcutaneous veins.

Changes in the volume of the hind limb were measured by a plethysmograph connected to a lever registering on a smoked drum. Vasoconstriction in the limb is indicated by a drop in the curve and vasodilation is indicated by a rise.

The graphic records (fig. 1) show distinctly that heating the center produces dilatation of the vessels of the hind limb and cooling the center causes vasoconstriction. Figures 2 and 3 show that these changes do not take place in the denuded limb.

CONCLUSIONS

1. Cooling the "heat center" in the corpus striatum in rabbits causes vasoconstriction in the hind limb.
2. Heating the center causes vasodilatation in the hind limb.
3. The skin vessels are the vessels mainly concerned in these effects, as shown by negative results with the limb denuded.
4. Further evidence is furnished of a central mechanism in the corpus striatum which exercises control over the vasomotor centers.

The expenses of the research reported in this paper were defrayed by the Loomis Medical Research Fund.

The authors desire to express gratitude to Dr. Yandell Henderson and Dr. H. G. Barbour for their advice and assistance.

BIBLIOGRAPHY

(1) Barbour: Arch. f. Exper. Path. u. Pharm., 1912, lxx, 1.
(2) Barbour and Prince: Journ. Pharm. Exper. Therap., 1914, vi, 1.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLVIII. Studies in Water Drinking

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In this work a study has been made (1) of the influence of copious water drinking with the meals upon gastric secretion and the emptying time of the stomach; (2) of the emptying time of water from the empty stomach; (3) of gastric stimulation by water; (4) of the latent period of the human gastric glands when stimulated by water; and (5) of gastric glandular fatigue.

WATER DRINKING WITH THE MEALS AND GASTRIC SECRETION

That water is a gastric stimulant has been pointed out by many investigators, e.g., Heidenhain (1), Sanotski (2), Pavlov (3), Krshyschkowshy (4), Foster and Lambert (5), Bergeim, Refuss and Hawk (6), Sawtch and Zeliony (7), Carlson, Orr and Brinkman (8). It has been shown by Foster and Lambert that water not only stimulates gastric secretion when given alone but that it also stimulates the gastric glands when it is given with food. Although, as Pavlov has shown, the stimulation is chemical, it has been demonstrated by Carlson, Orr and Brinkman that the psychic or thirst factor is of importance in the stimulation of the gastric glands by water. With this point in view the work of Foster and Lambert has been repeated.

Methods. Dogs with Pavlov accessory stomachs were prepared and put on a diet of 300 grams of meat and 800 cc. of water per day. The dogs were given two meals each day, one at 8.00 a.m., the other at 3.00 p.m. The juice was collected after each meal over a period of three hours. When water was given with the meal, the amount was 300 cc. An attempt to give the dogs, which were rather small, 500 cc. of water with the meal caused evident discomfort. So 300 cc. of water was the amount decided upon to be used in this series of experiments. In order to eliminate a possible factor of normal variation, the water

meals were varied; some days water would be given with both meals' then with neither or only with the morning or afternoon meal. The water when not given with the meal was given after the evening meal and at 11.00 p.m. The dogs were always offered water before the meal to see if thirst was present. It was refused in every instance. The water was always given by tube as the dogs would not drink any of it. The dogs, after training, took the tube without any apparent discomfort and would even allow the tube to remain in position indefinitely.

In table 1 the degree of acidity is expressed in clinical units (the number of cubic centimeters of N/10 NaOH required to neutralize 100 cc. of gastric contents). The peptic activity is expressed in millimeters of digested coagulated egg white, according to Schiff's modification of Mett's method. The length of the period of diet and experimentation was twelve days, so that the figures in the table represent the average of three days' results under each procedure.

The results upon these five dogs (table 1) show that there was always an increase in the amount of juice, but the increase in acidity did not occur in every instance. The amount of pepsin generally remained the same but often when the amount of juice was markedly increased, the peptic activity would be reduced. Although on a diet, these dogs showed some variation in the amount and acidity of the gastric juice from day to day, the peptic activity was more constant. By varying the procedure as described above, this factor of daily variation was eliminated.

It is apparent from these results that when factors of thirst and normal variation are controlled there is an increase in the total amount of gastric juice and in the free and total acidity of this gastric juice upon the ingestion of water with the meals, confirming the results of Foster and Lambert (5). It is interesting to note from the table that when the water was given with the afternoon meal, in every case but dog I, the amount of juice was increased over the amount when the water was given with the morning meal. The dogs were probably slightly thirsty, not having had any water between meals, although they refused water offered to them before the meal. It, however, must be recalled that the water was given by tube which is said to eliminate the psychic factor. Two explanations are possible: first, that the increased stimulation by water during thirst is not due to a psychic secretion but to an increased irritability of the gland cells to respond to the chemical stimulation of the water; or second, the psychic influence is not eliminated by the stomach tube because the sensation of cooling the throat,

oesophagus and stomach, which is present with the tube in, is still experienced. The factor of dilution of the blood as suggested by Carlson, Orr and Brinkman (8) may also be a factor concerned in this increase.

TABLE 1

Influence of water drinking with the meals on gastric secretion

		PROCEDURE	GASTRIC JUICE							
			No water with meal				H_2O with meal			
			Amount of juice 3 hours	Acidity		Pepsin	Amount of juice 3 hours	Acidity		Pepsin
				Free	Total			Free	Total	
			cc.			mm.	cc.			mm.
Dog I..	1	H_2O with morning meal	16	57	82	1.8	22	60	85	1.8
	2	H_2O with afternoon meal	13	37	72	2.0	19	65	97	1.8
	3	H_2O with both meals					24	60	87	2.0
	4	No H_2O with either meal	13	62	82	1.8				
Dog II.	1	H_2O with morning meal	20	72	107	1.5	34	97	110	1.25
	2	H_2O with afternoon meal	20	52	77	1.5	50	112	125	1.25
	3	H_2O with both meals					35	112	122	1.00
	4	No H_2O with either meal	21	75	102	1.5				
Dog III.	1	H_2O with morning meal	58	127	135	2.0	72	125	132	1.7
	2	H_2O with afternoon meal	60	117	125	2.0	75	125	130	1.7
	3	H_2O with both meals					71	120	130	2.0
	4	No H_2O with either meal	56	117	125	2.0				
Dog IV.	1	H_2O with morning meal	17	100	107	1.0	25	107	120	1.0
	2	H_2O with afternoon meal	18	97	105	1.0	28	105	122	1.0
	3	H_2O with both meals					24	105	120	1.0
	4	No H_2O with either meal	17	100	110	1.0				
Dog V..	1	H_2O with morning meal	24	95	105	2.0	35	100	115	2.0
	2	H_2O with afternoon meal	25	92	105	2.25	39	97	110	2.0
	3	H_2O with both meals					34	100	112	2.0
	4	No H_2O with either meal	23	95	105	2.0				

Water drinking with the meals and gastric stimulation: man. A series of experiments was next conducted to see if stimulation resulted in man upon drinking copious amounts of water with the meal. The man experimented upon was placed upon a diet of two meals a day, one at 11.00 a.m., the other at 5.30 p.m. The meal consisted of 125 grams of

graham crackers, 50 grams peanut butter, 300 cc. milk and 400 cc. of water. This was the amount of fluid normally taken during the meal by this person. The diet lasted eight days. Eight hundred cubic centimeters of water were ingested when the amount was called "copious." The "copious" water meal was varied so that two of the days the meals were of "moderate" water (400 cc.), or of "copious" water (800 cc.), or of "copious" water with the first meal or with the second meal. This was done for the purpose of eliminating the chance of any normal variation in gastric secretion. The factor of thirst was controlled by

TABLE 2

Subject I

TIME	400 cc. H_2O with meal		800 cc. H_2O with meal		REMARKS
	Acidity		Acidity		
	Free	Total	Free	Total	
Residuum 7.00 a.m.	17	22	30	40	Meal
8.00 a.m.	0	17	0	23	
8.30 a.m.	1	25	7	40	
9.00 a.m.	1	32	15	80	
9.30 a.m.	17	47	50	100	
10.00 a.m.	25	82	42	115	
10.15 a.m.			67	122	
10.30 a.m.	40	105	70	112	
10.45 a.m.	45	115	52	85	
11.00 a.m.	45	115	60	82	
11.15 a.m.	57	100	60	82	With 800 cc. stomach empty
11.30 a.m.	62	95	60	75	
11.45 a.m.	62	95	45	50	
12.00 noon	42	55	35	42	With 400 cc. stomach empty
12.15 p.m.	35	40			

keeping the daily amount of water intake constant and by drinking certain amounts at 7.00 a.m. and at 11.00 p.m. Samples of the gastric contents were withdrawn and analyzed for acidity. The emptying time of the stomach was recorded.

The following table 2 shows typical results obtained where 400 cc. and 800 cc. of water were taken with the meal.

Table 2 shows that there is only a slight increase in the free and total acidity of the gastric contents and that the rise in the acidity comes sooner and the stomach empties quicker when "copious" water is

ingested with the meal. This occurred in every case in this one individual.

This experiment was repeated on (Mr. F. V.) a gastric fistula case. The meal consisted of bread,[1] potatoes and coarsely ground meat mixed with milk to the consistency of a "mush," 300 cc. of this mixture being injected into the stomach via the fistula. The amounts of water injected after the injection of this food mixture were 150 cc. and 450 cc. The following table shows four typical results obtained.

The interchanging of the 150 cc. and 450 cc. water meals eliminates the possibility of a misinterpretation of the results. Mr. F. V. reports from personal observation that when he puts water into his stomach "the food leaves quicker." These results, shown in table 3, not only

TABLE 3

Influence of water drinking with the meal on acidity and emptying time

TEST	150 cc. H_2O with meal			450 cc. H_2O with meal			REMARKS
	Acidity		Emptying	Acidity		Emptying	
	Free	Total	Time	Free	Total	Time	
I	*87	112	1 hr. 25 min.	92	122	1 hr. 10 min.	
III	102	120	1 hr. 30 min.	110	127	1 hr. 15 min.	
IV	95	110	1 hr. 30 min.	97	125	1 hr. 10 min.	
VI	95	117	1 hr. 45 min.	100	122	1 hr. 30 min.	
VII	90	112	2 hrs.	112	127	1 hr. 40 min.	700 cc. used instead of 450 cc.
IX			1 hr. 50 min.			1 hr. 35 min.	700 cc. used instead of 450 cc.

* Acidities are maximum.

verify the subject's observation, but practically duplicate the results upon subject I (table 2). There is a definite increase in the acidity and a noticeable decrease in the emptying time of the stomach when the larger amount of water was taken with the meal.

Not being able to get other men to work upon, it was decided to repeat this work upon dogs and cats. The dogs were studied by means of gastric and duodenal fistulas and the cats by means of X-ray. The cats were fed 50 grams of salmon mixed with 10 grams of barium

[1] On two days the meals consisted chiefly of meat, Mr. F. V. substituting this without my knowledge. The emptying time of each meal with large and small amounts of water was two hours. In other tests the above meal was strictly adhered to.

sulphate. The dogs were fed 150 grams of finely ground cooked meat mixed with 50 cc. of water.

The first gushes of gastric contents appeared in from 15 to 45 minutes after the ingestion. Chyme appeared sooner in dog I and later in dog IV. The time for its appearance was fairly regular in each of the four dogs; in dog I, 15 to 20 minutes; in dog II, 20 to 30 minutes; in dog III, 30 to 40 minutes; and in dog IV, 35 to 45 minutes. Moritz (9) and Cannon (10) observed that the exit began about three-quarters of an hour after feeding, while Cohnheim and Lang (12) report that the exit begins in 15 minutes. Cannon explains the discrepancy to a difference in the consistency of the food. But here food of the same consistency was fed to four dogs and each showed a different exit time.

TABLE 4

Influence of water drinking with the meal on acidity and emptying time

DOG	50 cc. H_2O WITH MEAL			400 cc. H_2O WITH MEAL			REMARKS
	Acidity		Emptying	Acidity		Emptying	
	Free	Total	Time	Free	Total	Time	
I	100	132	3 hr.	105	137	2½ hr.	
II	77	122	3½ hr.	85	125	3 hr.	This dog two out of six trials showed no decreased emptying
III*	80	120	3½ hr.	80	120	3½ hr.	
IV	75	117	4 hr.	82	122	3½ hr.	

* This dog in no instance showed a decreased emptying time with 400 cc. H_2O. The dog may not have responded to water stimulation. It died before this could be verified.

Might not the discrepancy in the results of Cohnheim-Lang and Moritz-Cannon be explained by an individual variability in the exit time of the stomach in the dogs they worked upon?

The results obtained upon the cats were variable and unsatisfactory and do not warrant consideration.

These observations upon dogs and man suggest that the emptying time of the stomach is decreased by the ingestion of "copious" amounts of water with the meals. This confirms Cannon's (10) and Hedblom's observation that "the dilution of the protein food tends toward a more rapid discharge of the protein from the stomach." Attention must be called to the fact that in the experiment upon the dogs the water and meat was mixed before feeding while in man the water was taken at intervals during the meal, which would have a tendency to mix the

food and water and to retard the emptying of water from the stomach which, according to Cohnheim (12), does not take place during digestion. On this point, however, the literature does not agree as Leven and Barret (13) have found that, although water empties rapidly from the resting stomach, the discharge of water from the stomach when taken with food is retarded. Groebbels (14) states that bread followed by water shortens the time of the digestion of the bread. Gabrilowitch (14) states that when water is mixed with meat the water passes out and the meat follows the customary digestion.

The following table shows the results obtained for the discharge of 400 cc. H_2O from the empty and full stomach.

TABLE 5

Emptying time of water when taken into empty and full stomach

DOG	EMPTYING TIME OF 400 CC. H_2O FROM THE EMPTY STOMACH		EMPTYING TIME OF 400 CC. WITH 150 GRAMS OF MEAT IN STOMACH	
	Maximum	Minimum	Maximum	Minimum
I	45 min.	30 min.	1 hr.	45 min.
II	55 min.	40 min.	1 hr. 20 min.	1 hr.
III	1 hr.	50 min.	1 hr. 15 min.	1 hr.
IV	1 hr. 15 min.	55 min.	1 hr. 45 min.	1 hr. 25 min.

Time was taken when 400 cc. of fluid were obtained from the fistula. This, of course, contained particles of food and some gastric juice.

Three tests were made in each dog. The 400 cc. of water were given directly after the meat. Without delay water came from the duodenal fistula, but did not "pour" through the fistula as described by Cohnheim (12). It did, however, come in copious gushes of from 10 to 20 cc. which gradually decreased in quantity.

The above experiment was repeated upon Mr. F. V., a gastric fistula case. The normal emptying time of 450 cc. of H_2O from the empty stomach of this man was 15 to 20 minutes. When the same amount of water (450 cc.) was put into the stomach containing food the emptying time of the water from the stomach was 40 minutes,[2] as shown by

[2] It was impossible to make as accurate observations here as were made in the dogs. A judgment had to be made from the ease with which the sample was obtained from the stomach and from the absence of food in this sample. In collecting the samples Mr. F. V. places a small stiff rubber tube through the larger rubber tube, which is always kept in place; he then leans forward and the contents run out through the stiff rubber tube.

the average of five tests. In other words, with food in the stomach the emptying of 450 cc. of water was retarded 25 minutes.

The observations upon these dogs and upon Mr. F. V. support Leven and Barret's findings. There is always retardation, but the degree varies in different dogs and to a less extent in the same dog. This retardation in the emptying time of the water is unquestionably caused by the water mixing with the food, rendering the food more dilute. This being the case, then, the decreased emptying time of the stomach with "copious" water with the meal is due to the dilution of the stomach contents, facilitating digestion and evacuation.

EMPTYING TIME OF WATER FROM THE EMPTY STOMACH

The emptying time of water in those four dogs varied from 30 minutes to 1 hour. It varied slightly from time to time in the same dog. The water began to enter the intestine practically as soon as it reached the stomach, i.e., 5 to 10 seconds after it was introduced per os or per gastric fistula. In every dog it passed from the stomach in single gushes at varying intervals of from 10 to 30 seconds, each gush delivering from 5 to 30 cc. of water. These gushes as to time seemed to occur in groups, e.g., several of 10 second intervals would occur, then several of 15 seconds, then several of 12 seconds, and between these there might be interposed one or two of 5 seconds, or of 30 second intervals. There was evidence of rhythm. The gushes became slower as the stomach became empty. The water during the first few minutes was practically neutral, but after five minutes the acidity ranged from .02 per cent to .04 per cent and increased steadily.

These observations confirm those of Moritz (9) and London and Sulima (11) except as to the time between gushes. They report that the interval between gushes is too short to be accounted for by peristaltic movements. But from the observations made in this series of experiments the gushes could easily correspond to the occurrence of the peristaltic waves or stomach contractions, as reported by Von Mering (21) in 1893.[3]

In man, as will be seen from table 6, the emptying time of water rom the empty stomach also varies. In normal individuals, enjoying perfect health, the emptying time varied from 400 cc. in 15 minutes to 0 cc. in 15 minutes. The cases of gastric fistula, gastric ulcer and

[3] I believe that the operative technique is of importance in explaining the conflicting observations. See a former paper for a description of the methods used in making the duodenal fistula. (This Journal, 1918, xlvi, 340.)

gastro-enterostomy emptied 360 cc. in 15 minutes. The rate of emptying generally does not vary more than 40 cc. per 15 minutes period in the same individual when 400 cc. are drunk. There was one exception to this, however, occurring in subject W, table 6.

TABLE 6

Gastric stimulation by water in twenty men. Drinking 400 cc. H_2O

SUBJECTS	AVERAGE AMOUNT OF FLUID OBTAINED BY STOMACH TUBE AFTER 15 MINUTES	ACIDITY OF CONTINUOUS GASTRIC SECRETION	AVERAGE MAXIMUM ACIDITY AFTER INGESTION OF 400 CC. H_2O	REMARKS
	cc.			
I	300	25.0	60.0	Similar response to Ewald test meal
K.N.	280	30.0	70.0	Similar response to Ewald test meal
H.	270	55.0	95.0	Similar response to Ewald test meal
R.	340	45.0	115.0	
M.	200	47.5	62.5	
S.	200–230	7.0–22.5	52.5	Similar response to Ewald test meal
K.	355	20.0	55.0	Similar response to Ewald test meal
Se.*	400*	35.0	55.0–65.0	Similar response to Ewald test meal
Ma.	300	37.5	57.0	
N.	150	10.0	22.5	Responded to Ewald meal. Total acidity 40
W.	110–220	10.0–17.5	17.5–47.5	Quite variable: Six tests were made upon this person
C.	100	10.0	20.0	Responded to Ewald meal. Total acidity 50
L.K.	170	37.5	45.0	Responded to Ewald meal. Total acidity 50 to 60
O.	150	35.0–50.0	40.0–55.0	Responded to Ewald meal. Total acidity 50 to 60
G.	140	35.0	37.5	
Gr.	130	27.5	32.5	Responded to Ewald meal. Total acidity 50 to 55
F.	35	80.0	95	Gastric fistula
Mi.	30	80.0 (Contents)	105	Gastric ulcer
T.	300–400	40	30	Free acidity 10—Gastric carcinoma
J.	40	45	70	Gastro-enterostomy, 7 years standing

* This subject would withdraw from his stomach after drinking 400 cc. of water (at the end of 15 minutes) 415 to 420 cc. of contents. In other words withdrew from his stomach after 15 minutes more fluid than he put into it.

Holyknecht (16) states that 200 cc. of water are evacuated in 60 minutes, and Kastle (17) gives 70 minutes as the emptying time for the same amount. Ernst (18), Waldeyer (19) and Kauffman (20) have produced evidence of a "Rinne" or trough in the lesser curvature, which was also pointed out by Cohnheim (12), in case the stomach contains food. Scheunert (22) from experiments on the horse's stomach states that liquid in the distended stomach penetrates along the gastric wall. Von Mering (21) reports that practically 500 cc. of water are emptied from the stomach in 15 minutes. Bergeim, Refuss and Hawk (6) cite one case in man in which 500 cc. of water left the stomach in from 10 to 20 minutes. Such marked discrepancies in the literature are apparently explained by the wide range in individual variability pointed out by the series of results in this study on man and dog.

That water leaves the stomach much sooner than milk or food cannot be questioned; but it is not emptied as fast as Cohnheim's and Von Mering's observations would lead one to believe. As judged from this series the emptying of water from the normal human stomach varies, conservatively, from 400 cc. to 100 cc. in 15 minutes.

GASTRIC STIMULATION BY WATER. MAN

It has been shown by Bergeim, Refuss and Hawk (6) that the human stomach is stimulated by water and further they report that "in the average normal individual water produces fully as great a stimulation as does the Ewald test meal." While studying the gastric secretion of several normal men, it was observed that some of these failed to respond, or responded but poorly, to gastric stimulation by water and that these stomachs emptied water fast as compared to those that gave a marked response to stimulation by water. This observation has been extended and study has been made of the gastric secretion of twenty men, seventeen of them reporting normal gastric histories.

Methods. All of the tests were made in the morning before any food had been ingested and from two to three hours after the last drink of water. The stomach was emptied, the continuous secretion taken for two or three 15 minute periods, 400 cc. of cool tap water were drunk, the stomach completely emptied after 15 minutes and every 15 minutes thereafter for 2 hours. The stomach was not emptied at the end of 15 minutes if the results were to be compared with an Ewald meal, in which case samples were withdrawn for analysis. At least three tests were made upon each individual.

Figures 1 to 7 inclusive show the type of stimulation resulting in some of my subjects. In table 6 is recorded a summary of abstracted results of the emptying time of water from the empty stomach and the resulting stimulation in each of the twenty subjects studied.

The liquid taken from the stomach 15 minutes after drinking was generally bile-tinged (Gmelin's test), indicating either that some of it came from the intestine or that bile was regurgitated as the stomach was emptied for the bile appeared in the last portion withdrawn. The amount of liquid drawn from the stomach never varied more than 40 cc. in the same individual. It was observed that on warm days the

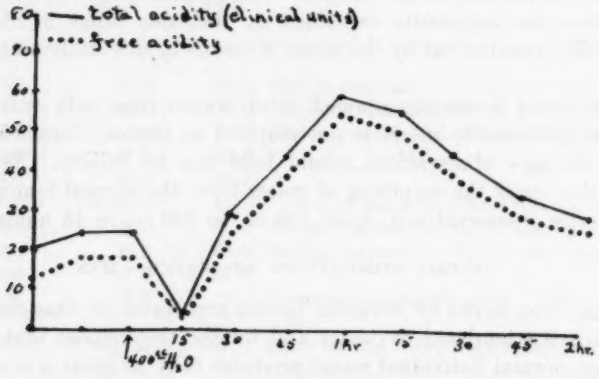

Fig. 1. Many tests were performed upon this subject. The response to water varied but little and was practically the same as was obtained with an Ewald test meal. On an average 300 cc. of water were taken from his stomach at the end of 15 minutes; 400 cc. were emptied in 1 hour.

water left the stomach quicker. This factor was controlled as well as possible. The mechanism of this observation was not studied. That the stomach was empty was always verified by blowing into the tube; if bubbles were felt, the stomach was not empty; if a swelling sensation was experienced, the stomach was empty. During the time of aspiration the body was rotated so that the tip of the tube would lie in the most dependent portion of the stomach cavity

The results upon the normal individuals indicate that the stimulatory power of water in the human stomach varies noticeably with different individuals, some responding markedly and others practically not at all. It is also apparent that those stomachs which empty the

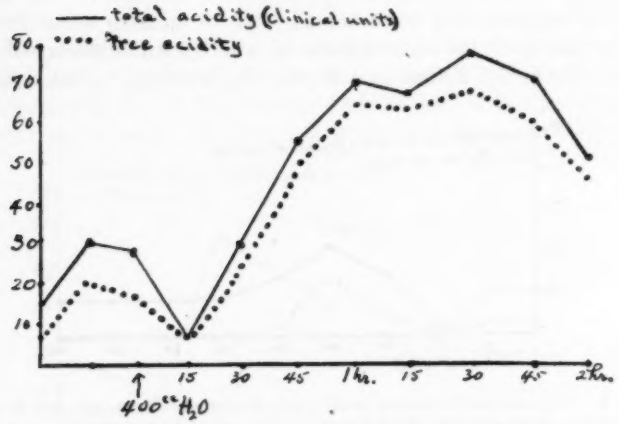

Fig. 2. This subject, K. N., always showed marked stimulation to water On the average 280 cc. were taken from his stomach at the end of 15 minutes

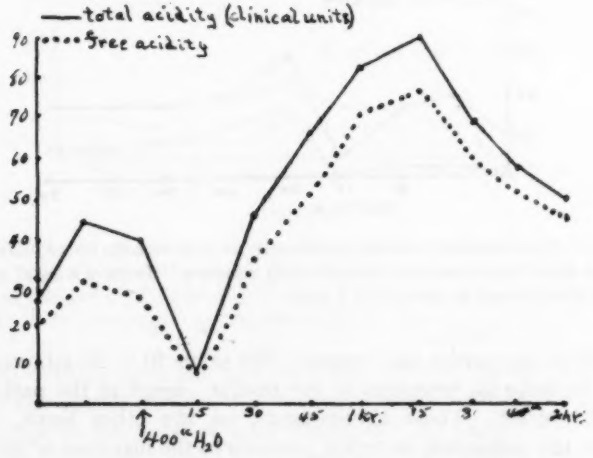

Fig. 3. The response here is also marked; 350 cc. were taken from his stomach at the end of 15 minutes.

water quickly do not respond to stimulation by water. The amount of water regained from the stomach after 15 minutes seems from the data in this study to be diagnostic of whether stimulation will occur or not. Bergeim, Refuss and Hawk (6), however, report a marked

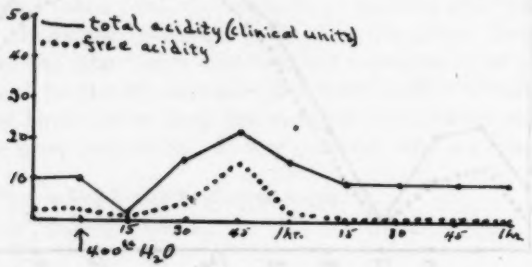

Fig. 4. This subject N shows practically no response to water, yet he gives a total acidity of 40 with the Ewald test meal at the end of an hour. Only 100 cc. of water were obtained from his stomach at the end of 15 minutes.

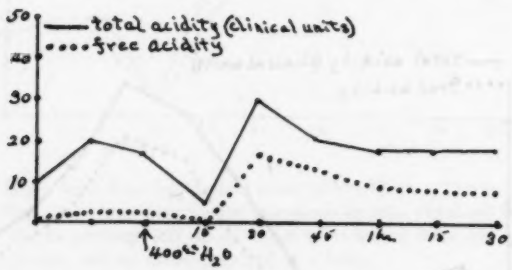

Fig. 5. This subject C shows practically no response to water; 100 cc. were obtained from his stomach at the end of 15 minutes. He gave a total acidity of 50 to an Ewald meal at the end of 1 hour.

response in one person who emptied 500 cc. in 10 to 20 minutes which would be quite an exception to my results, except in the pathological cases I present. These investigators, on the other hand, did not measure the continuous secretion previous to the ingestion of the water, which questions the validity of such a stimulation.

LATENT PERIOD OF THE HUMAN GASTRIC GLANDS WHEN STIMULATED BY WATER

Pavlov has shown that in dogs the latent period of the gastric glands when stimulated by water is 5 minutes. This has been confirmed in this laboratory by Dr. G. F. Sutherland and myself, working separately. The latent period may be as long as 15 minutes. Bergeim, Refuss and

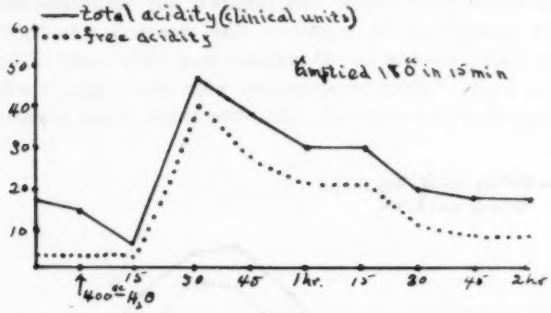

Fig. 6

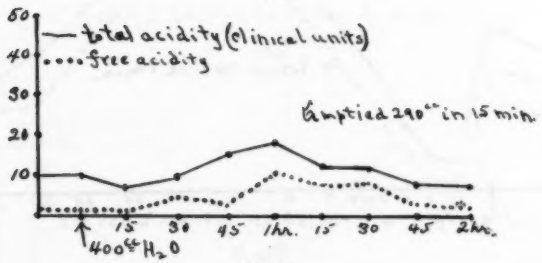

Fig. 7

Figs. 6 and 7. In subject W there was quite a noticeable variation in the emptying time. Figure 6 shows the gastric response when 220 cc. were taken from his stomach at the end of 15 minutes and figure 7 shows the response when 110 cc. were taken from his stomach at the end of 15 minutes.

Hawk (6) working upon one man state that "it was impossible to demonstrate any latent period for the human gastric glands." As some observations in the series of men suggested that there was a latent period and as Bergeim and his colleagues in their experiment did not take into consideration the continuous secretion of the stomach and used water for a lavage just before using it for a stimulus, it was decided to investigate the correctness of their conclusion.

It may be seen from the graphs in part 3 that the residuum is generally lower in acidity than the continuous secretion, due as suggested by Doctor Carlson to dilution by saliva and neutralization by ammonia (24). So the acidity of the residuum cannot be taken as the acidity of the continuous secretion. In this series of experiments, then, the stomach was emptied, the continuous secretion taken for three periods, 400 cc. of water were drunk and pumped out simultaneously. This never took more than 1½ minute. The stomach was then emptied completely every minute for 10 minutes and then every 5 to 10 minutes for 1½ hour. These experiments were done upon three men, in two by means of the stomach tube, the third being a gastric fistula

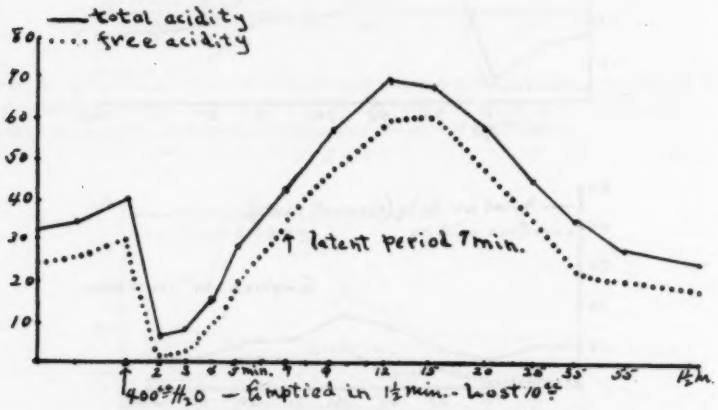

Fig. 8

case (Mr. F. V.) (23). Three tests were made upon the same individual. Figures 8, 9, 10 show typical results upon the three men used.

These graphs (figs. 8, 9, 10) show that there is a latent period of from 5 to 10 minutes when the human gastric glands are stimulated by water, which corresponds to Pavlov's findings in the dog. In subject I the latent period for meat broth was 5 minutes. In subject F. V. the latent period of the psychic secretion is from 4 to 7 minutes. (See Carlson: Control of hunger in health and disease, Chicago, 1916). It is apparent from figures 9 and 10 how absence of a latent period could be mistaken, if the acidity of the residuum was taken as the acidity of the continuous secretion.

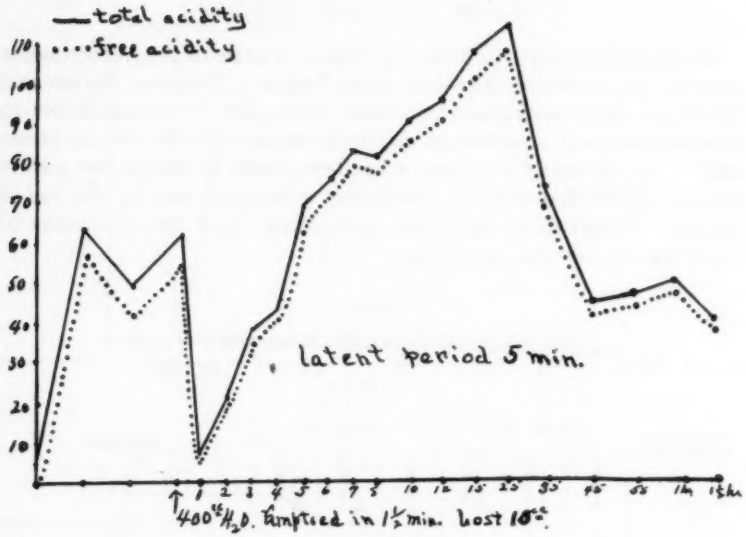
—total acidity
....free acidity
latent period 5 min.
400cc H2O. Emptied in 1½ min. Lost 18cc.

Fig. 9

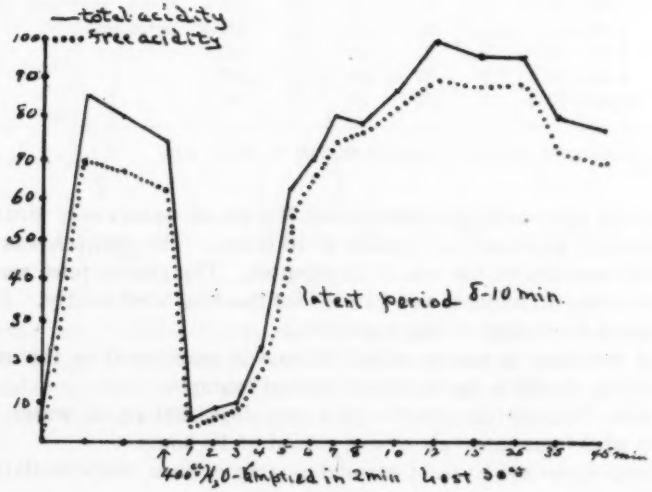
—total acidity
.....free acidity
latent period 8-10 min.
400cc H2O-Emptied in 2min Lost 30cc

Fig. 10

GASTRIC GLANDULAR FATIGUE

Foster and Lambert (5) from the results of some of their experiments suggest the presence of gastric gland fatigue. Bergeim, Refuss and Hawk (6), using water as a stimulant, state that "it is impossible to demonstrate any pronounced glandular fatigue in the human stomach." In this study an attempt has been made to fatigue the gastric glands of man and dog by stimulating with water and by the use of gastrin. Observations have also been made upon the possibility of fatiguing the psychic secretion.

TABLE 7

Gastric stimulation by water at two hour intervals

PROCEDURE 700 cc. H_2O	DOG I			DOG II			REMARKS
	Gastric juice			Gastric juice			
	Amount	Free acidity	Total acidity	Amount	Free acidity	Total acidity	
	cc.			cc.			
8.00–10.00	4.0	42	70	3	75	82	Dog had been kept from food and water for 24 hours previous to experiment
12.00	12.0	70	85	9	90	110	
2.00	10.0	75	87	12	95	112	
4.00	13	67	80	11	82	107	
6.00	11	70	85	13	87	112	
8.00	11.5	70	82	12	92	115	
10.00	12.5	72	80	10	90	110	

* Continuous secretion taken from 8.00 to 10.00 a.m.

In the first series of experiments 700 cc. of water were drunk at intervals of 2½ hours for a period of 10 hours. The stomach was completely emptied at the end of 15 minutes. The gastric juice was collected every 15 minutes and titrated for free and total acidity. Figure 11 shows the result of this procedure.

No evidence of gastric gland fatigue is manifested in the results obtained, of which figure 11 is a typical example.

Table 7 shows the results upon two dogs, 400 cc. of water being given at 2 hour intervals over a period of 10 hours.

There is no evidence of glandular fatigue when the stimulation is produced by water.

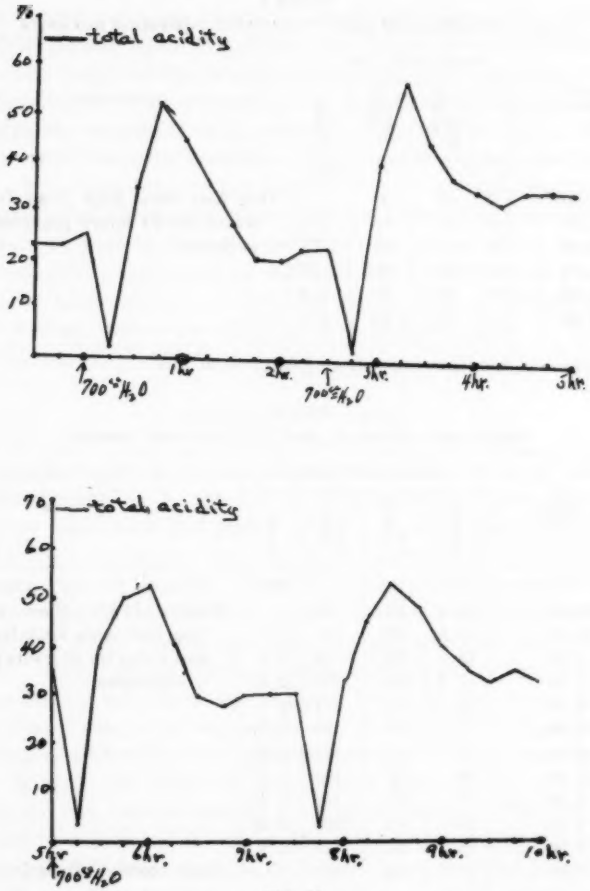

Fig. 11

It was next decided to attempt to produce fatigue of the gastric glands by injecting gastrin[4] every 2 hours over a long period of time. One cubic centimeter of gastrin was injected every two hours with the results shown in tables 8 and 9.

[4] Dr. F. C. Koch kindly furnished me with the gastrin used in these experiments.

TABLE 8

Gastric stimulation by gastrin infected at intervals of two hours

1 cc. GASTRIN PROCEDURE	GASTRIC JUICE: DOG I				REMARKS
	Amount	Free acidity	Total acidity	Pepsin	
	cc.			*mm.*	
8.00–10.00*	1.0	25	50		Dog had been kept from food and water for 24 hours previous to experiment
12.00	7.0	67	85	2.0	
2.00	8.0	67	85	1.75	
4.00	7.0	62	80	2.25	
6.00	8.0	67	87	2.0	
8.00	7.5	65	85	2.0	

* Continuous secretion taken from 8.00 to 10.00 a.m.

TABLE 9

Gastric stimulation by gastrin at two hour intervals

1 cc. GASTRIN PROCEDURE	GASTRIC JUICE: DOG II				REMARKS
	Amount	Free acidity	Total acidity	Pepsin	
	cc.			*mm.*	
8.00–10.00†	3.0	57	25		Acidity lower than normally
12.00	18.0	87	117	3.0	Dog had been kept from food and water for 24 hours previous to experiment
2.00	18.0	90	110	2.5	
4.00	17.5	90	105	2.75	
6.00	19	97	117	3.5	
8.00	21	90	110	2.75	
10.00	19	87	105	3.0	
12.00	20	87	105	2.75	
2.00 a.m.	17	87	102	2.25	
4.00	17	82	100	2.25	
6.00	19	85	110	2.5	
8.00	16	70	100		Juice bloody (digestion of skin about pouch)
10.00	17	75	102		Juice bloody (digestion of skin about pouch)
12.00	16	72	100		Juice bloody (digestion of skin about pouch)
Meal* 12.00–1.00	10	70	90		Juice bloody (digestion of skin about pouch)

* Normally the first hour of the meal produced 12 cc. of juice with free acidity of 90 and a total acidity of 115. The dog appeared to be very tired and did not eat with her normal degree of appetite.

†Continuous secretion.

Tables 8 and 9 are interpreted to mean that there is no fatigue of the gastric glands when stimulated by gastrin over a period of 26 hours. The slight reduction shown in table 9 is not significant because of the bleeding and the decrease in the acidity with the meal as compared with the normal is easily accounted for by the lack of relish of the food which was very manifest. This experiment is to be extended further. From the data obtained up to the present time the conclusion is warranted that the gastric glands are not fatigued by the continuous stimulation of gastrin during a period of 24 hours. Such a positive result is especially significant as during this period 240 cc. of juice were secreted by this method of chemical stimulation, although the dog had not had any water for 48 hours and had been kept in the stock during the period of collection.

GENERAL DISCUSSION

The question arises as to how this stimulation by water is produced. That the stimulation is not due to "the prolonged and widespread contact" of water with the gastric mucosa, as suggested by Pavlov, is shown by the stimulation occurring when the water is in contact with the mucosa only 1½ minute. Without deliberating one might explain by Pavlov's theory the results given in table 6, in which those stomachs that emptied water slowly gave the greatest response to stimulation by water. But it seems that the facts, as observed in this work, support the theory advanced by Carlson, Orr and Brinkman (8) who suggest that "the water washes traces of gastric secretogogues into the intestine, where they are absorbed and act on the gastric glands via the blood." For example, when water was put into the stomach and pumped out simultaneously 5 to 10 cc. were always lost, which could only be accounted for as having been emptied into the intestine along with gastric secretogogues from the stomach. The latent period of 5 to 10 minutes also suggests that such a mechanism exists. The observations made upon the relation of the emptying time of the stomach for water and gastric stimulation by water are also explained by this theory. For one would expect the secretogogues to be present in a higher concentration in the slow emptying than in the fast emptying stomach, as in the former there would be more time for digestion of mucin, food-remnants and of the proteins of the gastric juice and mucosa. Hence one would expect a greater stimulation by water in the slow emptying than in the fast emptying stomach. The fact that

fatigue of the gastric glands, when stimulated by water, cannot be demonstrated is due to the formation and collection of secretogogues in the rugae of the stomach, which are washed out by the successive ingestions of water, these ingestions of water being far enough apart to allow the formation of secretogogues in the meantime. The glands not being fatigued by gastrin, they would not be fatigued by secretogogues.

The dilution of the blood, as has been suggested, cannot be a basic factor in gastric stimulation by water for stimulation results, as shown by experiments on the latent period, when practically no water (not more than 10 cc.) is absorbed.

Whether or not the drinking of 400 cc. of water could be substituted for the Ewald meal in practice is an open question and will continue to be until a large series of observations has been made upon both pathological and normal individuals. According to the series presented in this paper, one would be led to believe that the Ewald meal cannot be substituted by water, because those individuals that responded but poorly to stimulation by water gave almost the normal response to an Ewald meal in every instance.

Further it is apparent from the curves shown in this paper that in all studies of gastric secretion, normal or pathological, experimental or clinical, the continuous secretion must be studied and taken into account. The stomach contents, or residuum, is not a true index of the continuous secretion.

One is not surprised to find marked discrepancies in the literature upon the emptying time of the stomach for both food and water when the marked normal individual variations are considered along with the many factors, both psychic and constitutional, that influence the activity and functioning of the organ. And it is evident from the observations made upon the twenty men in this series that the statements published in textbooks of physiology on the very rapid rate of the emptying of water from the stomach (500 cc. in 15 minutes) are misleading and false.

As to gastric glandular fatigue it is only reasonable to believe that as long as the normal gastric glands are supplied by the blood with the normal quality and quantity of constituents that form the gastric juice no fatigue will be observed, but as soon as the blood fails to supply these necessary constituents a change in the normal character of the gastric juice will occur. Even then it cannot be said that the glands are fatigued, but have only been deprived of the raw products

necessary for their normal functioning. The results of the experiments on the question of gastric glandular fatigue seem to bear out this idea. And it only remains to ascertain how long the secretion can be continued before the necessary constituents in the blood for the formation of gastric juice are depleted enough to produce a change in the normal character of the gastric juice, no food or water being supplied to prevent the depletion.

CONCLUSIONS

1. The ingestion of water with the meals (400 to 800 cc.) increases the amount and the free and total acidity of the gastric juice.

2. The ingestion of water with the meals decreases the emptying time of the stomach, due to the dilution of the stomach contents.

3. Food in the stomach retards the evacuation of water.

4. The emptying time of water from the normal human stomach varies, conservatively, from 400 cc. to 100 cc. in 15 minutes.

5. The manner of the discharge of water from the dog's stomach is, according to the observations upon four dogs, rhythmic and could very possibly correspond to peristaltic waves.[5]

6. All stomachs do not respond to stimulation by water, there being a marked variation in different individuals. Those stomachs that empty water slowly (150 cc. or less in 15 minutes when 400 cc. are drunk) respond much more than those that empty water fast. From the observations in this study water cannot be substituted for the Ewald meal.

7. The latent period of the gastric glands of man when stimulated by water is from 5 to 7 minutes.

8. It was impossible to demonstrate a fatigue of the gastric glands when stimulated by water or by gastrin for a period of 10 to 26 hours.

The writer desires to express his indebtedness to Doctors Luckhardt and Carlson for their valuable suggestions.

[5] In a personal communication, Dr. A. B. Luckhardt stated that this observation corroborates his findings upon which he has more definite data to be published later and which is referred to in a recent article by Dr. Carlson in this Journal, xlv, 87.

BIBLIOGRAPHY

(1) Heidenhain: Arch. f. d. gesammt. Physiol., 1877, 148.
(2) Sanotski: Arch. d. Si. Biol., 1892, i, 589.
(3) Pavlov: The work of digestive glands (trans. by Thompson), London, 1910.
(4) Krshyschkowsky: Dissertation, St. Petersburg, 1910.
(5) Foster and Lambert: Journ. Exper. Med., 1908, x, 820.
(6) Bergeim, Refuss and Hawk: Journ. Biol. Chem., 1914, xix, 345.
(7) Sawitch and Zeliony: Arch. f. d. gesammt. Physiol., 1913, cxl, 123.
(8) Carlson, Orr and Brinkman: This Journal, 1914, xxxiii, 91.
(9) Moritz: Zeitschr. f. Biol., 1901, xliii, 584.
(10) Cannon: Mechanics of digestion, 1911, 122.
(11) London and Sulima: Zeitschr. f. physiol. Chem., 1905, xlvi, 235.
(12) Cohnheim: Münch. med. Wochenschr., 1907, liv, 2581.
(13) Leven and Barret: Loc. cit.; Cannon: Mechanics of digestion, 1911, 122.
(14) Groebbles: Zeitschr. f. physiol. Chem., 1914, lxxxix, 1.
(15) Grabrilowitch: Ibid., 1912, lxxxi, 398.
(16) Holyknecht: Wiener klin. Wochenschr., xxxii and xxxiii, 1193.
(17) Kaestle: Rieder's Rontgenkunde, Leipsig, 1913, 532.
(18) Ernst: Loc. cit.; Cohnheim: Münch. med. Wochenschr., 1907, liv, 2591.
(19) Waldeyer: Cited by Groebbles.
(20) Kauffman: Zeitschr. f. Heilk., 1907, xxviii, 203.
(21) Von Mering: Verhandl. des Congresses f. innere med., 1893, xii, 471.
(22) Scheunert: Arch. f. d. gesammt. Physiol., 1912, cxliv, 411.
(23) Carlson: Control of hunger in health and disease, Chicago, 1916.
(24) Huber: This Journal, 1917, xlii, 404.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.
NO. 308

EXPERIMENTS ON THE NATURE OF THE SENSE OF SMELL IN THE COMMON CATFISH, AMIURUS NEBULOSUS (LESUEUR)

J. M. D. OLMSTED

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1. INTRODUCTION

The question as to whether fishes possess a sense of smell separate and distinct from that of taste has for several species been answered in the affirmative (Parker, '10, '11, '13; Sheldon, '11; Parker and Sheldon, '12). Experiments on the common freshwater cat fish, Amiurus nebulosus, have been especially successful, and for this reason and because of its availability and comparative hardihood in the laboratory, this species was chosen for the work the results of which are given in this paper. My acknowledgments are due Dr. G. H. Parker for suggesting the problem and for helpful criticism throughout the work.

2. MATERIAL AND METHOD

The younger fish were found to be best for experimental purposes since they became accustomed to laboratory conditions more quickly and gave more uniform reactions than the older ones. It was discovered early in the experiments that a single specimen of Amiurus in a tank does not respond normally, if at all, to olfactory stimuli but that at least two individuals must be kept together. This gregariousness, which is so characteristic of the species, has already been commented on by Parker ('10) and by Parker and van Heusen ('17a, b). It takes generally some three weeks of residence in the laboratory before the fish become accustomed to the frequent changing of water, the presence of the investigator, etc., and since these experiments were concerned with the reactions of Amiurus to food-materials, the fish had to be brought into a receptive state toward food and were

therefore left unfed during this period of readjustment. As soon as they nibbled eagerly at a bag containing minced earthworms, experimentation could proceed. The fish were kept, in some cases as long as five months, in good physical and experimental condition by feeding each individual daily half of a living earthworm.

The method chiefly employed in these experiments was the same as that of Parker ('10, '11) and of Parker and Sheldon ('12), viz., the suspending—in a tank containing the fish—of cheesecloth bags in which were concealed the materials to be tested. In a few cases the bags were attached to wires hanging from a wooden frame but after the discovery of the exceeding sensitivity of Amiurus to metal rods (Parker and van Heusen, '17a), cotton strings were employed in their place. In every trial two bags were used, one a dummy, containing merely a wad of cotton weighted with a small stone, the other the test-bag, exactly similar in appearance to the dummy, but containing some substance to be tested wrapped within the cotton wad. Rarely did the fish bite the dummy (cf. Parker, '11), yet in a few cases thos individuals which had been subjected to experimentation for several months would, during a trial, give two or three nibbles on the dummy, but this occurred only when their barbels touched the dummy, the fishes having been roused to swim by a stimulating substance in the test-bag, which they had not as yet located. They evidently learned to associate olfactory stimulation with the presence of a bag containing food and whenever they discovered any such bag by means of the sense of touch in their barbels, they bit at it. A very few bites on the dummy were sufficient, for the fish would then resume their search until they found the test-bag and once having found it, the numerous bites and often vigorous tussle left no doubt in the mind of the observer that, from the standpoint of olfactory stimulation, the few nibbles on the dummy-bag might be disregarded. If, however, the test-bag received no more attention than the dummy, viz., an occasional feeble bite, by a long trained fish, this was taken as proof that the substance in the test-bag was non-stimulating.

Further instances of the ability of Amiurus to learn were afforded by several specimens which would come to the surface of the water to receive bits of worm, etc., from the forceps or fingers, when the investigator waved his hand over the water. These fish would finally nibble at anything presented to them at the surface of the water, bare forceps, finger, paper, even pebbles, but when dummy and test-bags were placed on the bottom of the tank and the disturbance due to the lowering of

the bags had ceased, the fish bit at the test-bag only when it contained some substance which proved to be also stimulating to other fish, and paid no attention to the dummy.

In some cases, e.g., with blood, the test-bag became colored and therefore different in appearance from the dummy; but upon observation it was quite evident that sight plays little part in the food reaction of Amiurus (cf. Parker and Sheldon, '12). Herrick ('02) found that catfish did not "remember the color of cotton," and a small piece of brick, the color of raw meat, failed to attract them though they bit eagerly upon it after it had been soaked in meat-juice. In the present experiments most conclusive evidence upon this point was afforded by the behavior of blinded fish (eyes totally removed). These blind fish appeared to become aware of the presence of food even more quickly than the normal fish, perhaps because their unceasing restlessness kept them awake, as it were, and receptive to stimulation while the normal fish at rest—the condition at the beginning of each experiment—were rather sluggish and required to be "awakened" by the stimulus. The blind fish located food in practically the same time as the normal fish. It is well known that Amiurus is more active at night than in the day (Parker and van Heusen, '17b), being in nature a night feeder (Herrick, '02).

A test-bag was never used for more than one material, a new bag and fresh cotton being substituted at each trial. If a material proved to be stimulating to a fish, the water in the aquarium was changed after the trial, the aquarium scrubbed and rinsed and another trial was not made until at least two hours had elapsed.

The two bags were let down as gently as possible into the tank while the fish were at rest. This could generally be accomplished without disturbing the fish further than momentarily decreasing their rate of respiration. When a test-bag containing a stimulating substance, such as minced earthworms, was placed some eight inches from the head of a fish, the following sequence of reactions usually occurred: (1) The respiration rate returned to normal after about one minute, i.e., to what it was before the disturbance created by the lowering of the bags. (2) After remaining normal for about two minutes, the rate of respiration increased. (3) The large barbels at the corners of the mouth would then begin to twitch, (4) and after a very few seconds the fish would give a huge gulp, (5) lunge forward, apparently spasmodic, and (6) finally swim about searching along the bottom of the tank. If the dummy-bag alone was placed in the tank there was no

such behavior, the fish often remaining motionless for ten, fifteen or sometimes thirty minutes. The time elapsing between the lowering of the bags and the swimming of the fish varied greatly in the different trials and the variation seemed to be due as often to the individuality of the fish as to the nature of the substance tested. Substances only slightly soluble, such as dry blood albumin, did take longer to reach the fish, though when this particular material was previously soaked in water and the solution absorbed by the cotton used, the reaction time was the same as for other substances, viz., four to five minutes.

If, during the first fifteen minutes after introducing the bags into the tank, the fish reacted to the material in the test-bag by nibbling, nosing the bag or searching vigorously in its vicinity, the position of the two bags was reversed and observations were taken for another fifteen minute period. If, however, there was no reaction during the first fifteen minutes, the bags were left undisturbed for fully thirty minutes. Almost never did a trained fish fail to respond to a simulating substance in less than ten minutes, so that after the experimenter had learned the characteristics of a particular fish, fifteen minutes was often enough to indicate the efficiency of a substance as a stimulating agent. For purposes of comparison frequent trials were made with bags containing minced worm and unless the fish reacted readily and vigorously to these the previous sets of experiments were discarded.

A second form of experiment, employed with liquid substances, confirmed the results of experiments with bags. Two fish were placed in shallow dishes of water and when they had become perfectly quiet a pipetteful of the liquid to be tested was gently released above their anterior nasal apertures. If worm-juice or other stimulating material was used, the same series of reactions took place as with the bags, viz., increase in rate of respiration, twitching of the large barbels, a gulp and a lunge forward, but all these reactions occurred in very rapid succession. When a non-stimulating substance, such as water, was used, the fish usually showed irregularity in their rate of respiration but no other response followed. If the anterior nasal apertures of the fish were closed by sewing (Parker, '11), the only reaction, even to worm-juice, was a slight change in the respiration, and in many trials even this was not observable.

Table 1 gives a list of substances used in preliminary tests and their relative values as stimulating agents. To determine these values a record was taken of the number of bites and vigorous nosings made by two fish during thirty minutes. To that substance which caused the

greatest number of bites was assigned the value 100, and the others estimated accordingly. Since some thirty different pairs of fish were used in the experiments, at different seasons of the year, and, as indicated above, each fish seems to possess more or less of an individuality

TABLE 1

Effectiveness of various materials as olfactory stimuli to Amiurus nebulosus—based on the number of bites made by two fish during half-hour trials. The most stimulating materials are graded 100 and the others in proportion

WORMS	
A Lumbricus	
Whole	90
Minced	100
Decayed	100
Rotten	0
Dried	90
B Eisenia	
Minced	80
Decayed	80
BEEF LIVER	
Fresh	90
Stale	100
LEAN BEEF	
Fresh	20
Stale	60
Decayed	0
Dried	0
Stero-cubes	0
Commercial peptone	0
BLOOD	
Frog	20
Human	30
Sheep	60
Beef (fresh)	60
Beef (decayed)	0
Worm	75
MUTTON	
Fresh	10
Decayed	0
FROG MUSCLE	
Fresh	0
Decayed	0
AMINES	
Prophylamine	0
Higher amines (1)	0
Higher amines (2)	0
FISH MUSCLE	
Amiurus, fresh	0
Amiurus, decayed	0
Herring	0
Mackerel	0
SLIMY SUBSTANCES	
Frog's eggs	20
Limax	15
Necturus' slime	15
Human saliva	40

of its own, and all could certainly not have been in exactly the same physiological state as regards food, several of the values calculated on the basis indicated have been slightly changed in order to make the relative values consistent throughout all the tables.

3. GENERAL RESULTS

It has been intimated that this fish, Amiurus nebulosus, is more of less of a scavenger and it is a tradition, at least among rural fishermen, that rankly smelling substances, such as badly decayed meat or worms, are even better bait than fresh meat and fresh worms. Dean ('91) remarks that Amiurus nebulosus is an objectionable neighbor since fish eggs are often found in its stomach. "Nor is it fastidious in its diet, 'from an angleworm to a piece of tin tomato can', it bolts them all. . . . Professor Goode has already noted the attractiveness of salt mackerel for herring bait." Forbes and Richardson ('08) found that the food of this fish consisted of "small bivalve mollusks, larvae of insects, distillery slops and accidental rubbish." One specimen contained eighteen leeches.

An examination of table 1 will show that the results of my experiments rather disagree with the idea of "un-fastidiousness" in Amiurus nebulosus in its choice of food. With the exception of decaying earthworms, which will be discussed later, no putrefying material proved at all attractive to the fish nor was I successful in getting bites or even nosings upon bags soaked in several of the lower amines, though they most decidedly possess the characteristic odor of decaying meat.

The difference in behavior between catfish in the laboratory and those in a pond or river is readily accounted for when one realizes that an olfactory stimulus renders the fish aware of food and releases the impulse to seek for it, but that the food itself is usually located by the sense of touch (and perhaps taste) in the barbels after contact with it. In the tank there were only two cloth bags to interrupt the smooth glass surface, but in the natural habitat of the fish there are all sorts of objects over which the barbels are dragged. A very "hungry" fish will often nose quite inedible things, such as a pebble, and in one case I observed a fish, which had been roused to the seeking reaction by a bag of minced worms, take into its mouth its own faeces, only to cast them out immediately. So in nature hungry fish may, during their restless movements at night, swallow many things, e.g., salt mackerel and herring bait or even a bit of tin can, which do not stimulate the sense of olfaction.

But since human saliva proved to be fairly stimulating to the fish, the time-honored custom of "spitting on one's bait" does seem to be more than superstition, and perhaps for this reason may receive the sanction of science.

Among the substances tested, earthworms, beef liver and blood seemed to be the most promising for study.

4. THE COMPONENTS OF EARTHWORM MATERIAL AS STIMULATION AGENTS

It was rather astonishing to find that a living, uninjured earthworm, Lumbricus terrestris, although carefully wrapped in a wad of cotton and so tied in a cheesecloth bag that no movement was discernible, should be discovered as quickly by Amiurus and should prove to be practically as stimulating as small pieces of chopped fresh worm from which the blood and body-juices could readily escape. After the trials the worms were carefully examined and though the bags had been bitten, often as many as thirty times, the worms were found to be uninjured and their epidermis intact. The slime thrown off by Lumbricus as it travels unmolested over and through moist cotton is only half as stimulating as the worm itself but if a specimen on cotton is prodded and mauled, though not so severely as to injure its epidermis, this slime is equal in its stimulating power to the worm itself. It was noticed that when the worms were thrown into water of the same temperature as that in which the fish were kept, they writhed about much like the prodded worms on cotton. It seemed therefore that the ordinary products of the slime glands were not responsible for this attraction of the fish for whole worms, but that some further secretion, perhaps fluids from within the body-cavity escaping through the dorsal pores, were the stimulating agents. A quantity of ropy slime sufficient for experimental purposes was thrown off by living worms placed in water at 28° to 30°C. or in dilute (10 per cent) alcohol. Such slime, both before and after filtering, is exceedingly stimulating to the fish. Nevertheless, when this fact is taken into consideration with the results of trials on human saliva, Necturus slime, Limax and frog's eggs, one might naturally infer that the source of stimulation in these materials is that chemical substance common to them all, mucin.

The glycoprotein, mucin, is a relatively stable protein, not coagulated by heat, soluble in dilute alkali and precipitated by dilute acid. Slime from one hundred living earthworms in 250 cc. of water kept at 30°C. for thirty minutes, after being filtered, twice precipitated, washed and dissolved, and again precipitated and washed, yields a very small amount of grey slimy mucin, which when dried can be ground to a dirty yellow powder (cf. Hammarsten, '14, p. 170). This dried mucin does not dissolve in tap-water, and is not stimulating to Amiurus in this state. But even when dissolved in 1/1000 M KOH (an OH concentration which is not stimulating to Amiurus), it caused only the slightest reaction on the part of a few fish and none at all on the ma-

jority of those tested. Mucin prepared from human saliva (Hawk, '14, p. 123) or from the salivary glands of sheep, either dry or dissolved, failed to attract the fish. If sheep mucin is washed free from acetic acid after precipitation and immediately smeared on the outside of a bag, the fish will nibble at such a preparation but only when their barbels come into direct contact with the slimy material.

TABLE 2

Effectiveness of preparations from worms (Lumbricus terrestris) as olfactory stimuli to Amiurus

1. Whole worms	
Living, uninjured	90
Dried	100
2. Worm slime	
Cotton traveled over by worms	50
Cotton traveled over by prodded worms	85
Slime from worms in water at at 30°C. or in 10 per cent alcohol	95
Ether extract	0
Ether residue	85
Chloroform extract	0
Chloroform residue	25
Heated to 60°C	60
Heated to 70°C	0
Open to air four days	0
Preserved one week with thymol	40
Mucin	5
3. Minced fresh living worms	
Entire product	100
Filtrate	100
Residue from filtrate fresh	100
Same dried	100
4. Red filtrate from fresh minced worms	
Albumins	25
Globulins	25
Peptones	25
All proteins precipitated by acetone	100
Acetone residue from above	60
5. Ether extraction	
Fat	25
Residue fresh	100
Residue dried for months	100
6. Boiled	
Liquor	100
Acetone precipitate from liquor	100
Acetone residue from above	80
Worm bodies	100
Worm bodies dried	100
7. Decayed	
Without treatment	100
Alcohol extract	0
Alcohol residue	90

It is evident, therefore, that mucin is not the chemical substance which causes Amiurus to bite upon bags in which are concealed whole, living worms; mucin does, however, seem to stimulate the barbels when they come into direct contact with it.

There are indications that the substance or substances in the filtered slime of Lumbricus which attract Amiurus are of the nature of proteins.

(1) If the slime is shaken with ether, the two layers separated and the ether evaporated from each layer before an electric fan, the ether extract (on filter paper) has no attraction for the fish and the water residue loses some of its stimulating power. If the slime is similarly treated with chloroform, not only does the chloroform extract not stimulate the fish but the water residue becomes much less stimulating. If the slime is boiled or even heated to 70°C. it loses its stimulating power. Heating to 60°C. for three minutes slightly impairs this stimulating power but heating to 50° or less has no effect. Ether, chloroform and heat are general coagulating agents for proteins (Hammarsten, '14, pp. 97, 107). (2) If the slime is allowed to remain open to the air for several days and putrefaction takes place, it becomes non-stimulating. When preserved with a few drops of thymol solution, it retains somewhat of its stimulating power. Yet the ordinary chemical tests for proteins, Biuret, Millon's, xanthoproteic, etc., applied after the removal of mucin give only negative results. This probably means that the concentration of these substances is so low that they can not be detected by the ordinary color tests. If this is true, one of the characteristics of the olfactory sense of Amiurus is its ability to respond to exceedingly small amounts of substance, and this is in accord with what we know of the human sense of smell (Parker, '12).

Proof that the sense of smell alone is concerned in the reactions of Amiurus to worm slime is afforded by the behavior of fish with their barbels removed, and therefore lacking the majority of their gustatory organs (Parker, '10). These barbel-less fish find bags soaked in the slime only slightly less readily than normal fish. And in addition, fish with their olfactory apparatus temporarily eliminated by the sewing of their anterior nasal apertures, fail to pay any attention whatsoever to such a bag. A slight exception to this behavior occurs when an operated fish which has been subjected to experimentation for several months happens in its movements to touch the bag. Such a fish may give one or two gentle nibbles upon the bag. This is evidently nothing more than a matter of learning as previously described. or the response of a very "hungry" fish to tactile stimulus.

When living worms are passed through a meat-grinder and then allowed to soak in water for twenty-four hours, using thymol as a preservative (Wodehouse and Olmsted, '17), a clear red solution is obtained, which is as stimulating to Amiurus as the pieces of worm themselves. Upon separating these water-soluble proteins into globulins, albumins, peptones, etc., somewhat according to the scheme

outlined by Hawk ('14, p. 123), there were obtained preparations which, in either the moist or dry state, were always much less attractive to the fish than the original solution. The same was true of proteins soluble in salt solution. The insoluble residues from the water or salt extraction were always stimulating in either the moist or dry state. If, however, the red water-solution was filtered off immediately after grinding the worms and the proteins precipitated as a whole by acetone, this aggregate of proteins was very stimulating to the fish. But any attempts to separate them by redissolving, partially and wholly, saturating with ammonium sulphate, dialysing, etc., reduced their effectiveness almost to zero.

It was stated above that if the slime from Lumbricus was boiled or even heated to 70°C. it lost its attractiveness for Amiurus. This is not true of the worms themselves. Both the worms and the liquor in which they are boiled are as stimulating as fresh raw worms. If such boiled worms are dried, they lose none of their stimulating power even after six months.

When the proteins in the liquor from boiled worms are precipitated by acteone, a slimy grey coagulum is obtained, which is very stimulating to the fish, and even the acetone filtrate, after being evaporated to dryness on the steam-bath, is only slightly less so. If, however, alcohol is used as the precipitating agent, the coagulated proteins are only slightly stimulating while the alcohol filtrate is as strongly stimulating as the original solution. Likewise an alcohol solution made by placing living worms in 95 per cent alcohol until they become hard and brittle, is exceedingly stimulating, but an alcoholic solution made from decayed worms is non-stimulating though the residue of worm bodies, having lost much of its disagreeable odor, is stimulating. Chemical tests on the alcoholic solutions which prove to be stimulating indicate, but only very faintly indeed, the presence of a peptone. But doubt is thrown on the assumption that this is really the chemical substance responsible for stimulation since worm peptone prepared by several methods was hardly at all stimulating. This may again be interpreted as indicating the presence of exceedingly small amounts of stimulating substances, perhaps of the nature of proteins (since they seem to be destroyed by putrefaction), but in this case non-coagulable by heat.

If living worms are allowed to dry without putrefaction, they are found to be exceedingly stimulating to the fish, even after a year in this condition. The fat of freshly dried worms, extracted with ether in a Soxlett apparatus, is yellow and closely resembles ordinary animal

fat in appearance and properties. Such worm fat is only slightly attractive to Amiurus, showing that the very stimulating acetone and alcohol soluble substances mentioned above are not fats, and this is further supported by the fact that the hard dry pieces of worm remaining after the ether extraction are exceedingly stimulating.

Proof that these substances are not of the nature of volatile etherial oils is given in the results of the following experiments. Freshly ground living worms were placed with water in a flask provided with two openings. Through one of these openings air was forced up through the mixture and out through the other into a tube with a capillary outlet. This outlet was situated at the bottom of a cylinder of distilled water cooled by ice. Although air was bubbled through the worm mixture for twenty-four hours, the cooled water did not take on any peculiar or distinctive odor and such water was non-stimulating to the fish. A similar procedure when carried out on decaying worms did impart to the cooled water the characteristic odor which accompanies putrefaction, but this liquid was likewise non-stimulating. In one case a bag of minced earth-worms which had been left in running water for over a week without a preservative developed an almost intolerable odor and in this condition had no attraction whatsoever for the fish. Very completely decayed worms from which the water soluble substances have been practically entirely removed are therefore non-stimulating.

These results show that it is not the decayed material—the broken-down proteins—which stimulates Amiurus, and had the so-called "decayed" worms been thoroughly putrefied, they would probably have failed to excite the fish. In other words, the stimulus which caused Amiurus to react to "decayed" worms came from the still undecayed, unchanged substances.

Both fresh and decayed Eisenia foetida were stimulating to Amiurus, but some ten minutes after the fish bite these bags, they appear to be both attracted and repelled by the dung-worms. In a short time the repulsion becomes stronger than the attraction, the fish, on coming into the immediate neighborhood of the bag, dash wildly about the aquarium, jerk their heads away and often back off most vigorously. For this reason no extended experiments were carried out with Eisenia material.

5. EXPERIMENTS WITH BEEF LIVER AND BLOOD

The similarity of the results of experiments with earthworms, beef liver and blood will be evident upon comparing tables 2 3 and 4. It wil be noted that in no case was there found any component of the origina' protein mass that was not decidedly less stimulating than the original combination.

Fat was only very slightly stimulating.

If the watery extract from fresh liver is filtered and allowed to remain over night, a heavy brown coagulum settles out. This coagulum is slightly more stimulating even than the original water extract and possesses the odor of stale liver, an odor quite distinguishable from that

TABLE 3

Effectiveness of preparations from beef liver as olfactory stimuli to Amiurus

MINCED LIVER		FILTRATE FROM FRESH MINCED LIVER	
Fresh	90	Albumins and globulins together	30
Stale	100	Globulin	20
Filtrate from fresh	80	Albumin	15
Coagulum from fresh filtrate	100	Peptone	0
Dried coagulum	20	All proteins precipitated by acetone	0
Dried coagulum redissolved in water	60		
ETHER EXTRACTION		**BOILED LIVER**	
Fat	0	Liquor	100
Residue dry	80	Residue	90

of fresh liver. From table 1 it will be seen that stale liver is slightly more stimulating than fresh. Nevertheless ice-cooled water through which air was bubbled after having been previously forced through ground stale liver, and which possessed most distinctly the odor of stale liver, utterly failed to stimulate the fish. It seems evident, therefore, that the substance which gives stale liver and this coagulum from the watery extract of fresh liver the characteristic odor which distinguishes them so markedly from fresh liver, is not the same substance that renders them more attractive to Amiurus than fresh liver.

A further instance of substances possessing a very similar odor to the human sense of smell but differing markedly in their effect on the fish, is shown in the residue after ether extraction of stale beef or commercial

dried beef. Only the former of these two products stimulates Amiurus to bite.

The most striking differences in the results of the experiments on earthworms, beef liver and blood are: (1) Of the three kinds of material, earthworm alone was stimulating when "decayed,"—but other experiments show that the products of putrefaction are not the stimulating agents, (2) earthworm proteins alone retain their stimulating power after having been precipitated by acetone. This causes one to doubt whether such proteins (the globulins, albumins, peptones, etc., which can be detected and separated from each other by the ordinary chemical methods, and might be called the "gross" proteins) are really, even in the case of the earthworm, the stimulating agents for olfaction in Amiurus. Is it not more probable that in all these materials, substances which are present as "chemical traces" only, are responsible for

TABLE 4

Effectiveness of preparations from beef blood as olfactory stimuli to Amiurus

BLOOD AS WHOLE		DEFIBRINATED PLASMA	
Fresh	60	All proteins precipitated by acetone	0
Decayed	0	Albumins	0
Fibrin	0	Globulins	0
Defibrinated plasma	60	Peptones	0
		Ether extract	0
		Ether residue	0

the reactions of the fish? Some of them were shown to be destroyed by heat, others not; some are soluble in acetone and alcohol, others are rendered inactive by these reagents; they are not of the nature of fats or volatile oils; nor are they the products of the decomposition of proteins, in fact putrefaction seems to destroy them. In view of these characteristics it seems possible that they may be of the nature of proteins themselves.

The responses of Amiurus to stale liver and to the residue after extraction with ether were shown to be due to olfactory stimulation alone since fish with anterior nasal apertures sewn (with the exception of long trained fish) always failed to find bags of such material, while if the stitches were cut and the threads still left in the skin, the fish found the bags as quickly and bit on them practically as often as before the operation.

6. EXPERIMENTS WITH CHEESE

Fishermen have informed me that cheese, especially Limburger, is excellent bait for catfish. Table 5 gives the results of experiments on the effectiveness of this commodity as a stimulating agent, and shows that even Limburger cheese is not especially stimulating.

TABLE 5

Effectiveness of cheese as olfactory stimulus to Amiurus

AMERICAN		LIMBURGER	
Fresh	0	Fresh	10
Stale	0	Ether extract (rancid fat)	5
		Ether residue (protein)	5

7. THE ACTION OF THE BARBELS

A trained fish with its anterior nasal apertures sewn does not respond to bags of minced worm gently lowered in front of it but if the bag is carefully drawn toward the fish, the moment it touches a barbel, it will be seized by the fish. Rarely does this occur with a dummy-bag and never with a glass rod. This, again, seems to be a matter of learning, in which the sense of touch is concerned. The fish learn to distinguish the "feel" of the bag, which so often contains food. That the barbels do render service in food-getting is shown by the records of three pairs of fish whose reactions were followed for several months. From one fish of each pair all the barbels were removed and almost without exception this fish bit fewer times than its mate during the seventy odd half-hour trials. It was roused to action by an olfactory stimulus as soon as the other but was less able to locate the source. Parker ('10) describes the behavior of such fish as follows:

> Those without barbels, but with their olfactory apparatus intact, almost always made several sharp turns when near the wad (of minced worms) as though seeking something, and either moved slowly away, or swam more or less directly to the wad and began to nose and nibble it.

When a normal catfish receives an olfactory stimulus, it swims about with its barbels dragging along the bottom of the tank. The fish almost invariably stops for a moment if its barbels touch any object. If this object is edible the fish may nibble or swallow it, but if inedible the fish, after nosing the object, usually resumes its inter-

rupted search. It appears, therefore, that the barbels are very valuable to Amiurus in procuring its food but it receives stimuli through these organs only when they are in contact with a relatively large amount of material (cf. Herrick, '02, p. 257). The small particles of (presumably) molecular dimensions diffusing through water from a bag of minced worms cause no reaction in a fish whose olfactory organs are incapacitated to receive such stimuli. But if the barbels of such a fish come into contact with the bag, the fish *may* give a bite. If, in addition to the sense of touch, that of taste is involved in this reaction,—and Parker ('08) has shown that Amiurus possesses a gustatory sense located in its barbels,—then this sense in Amiurus is comparable to the human sense of taste (Parker '13), since a relatively large amount of substance locally applied is necessary to stimulate our organs of taste.

SUMMARY

1. Amiurus nebulosus bites readily on bags of earthworm, beef liver and blood.

2. The responses to these substances arise from the stimulation of the olfactory organs, as is proven by lack of responses when the olfactory apparatus is eliminated, and by the facts that blinded fish find this food as readily as normal fish, and barbel-less fish only slightly less so.

3. The barbels are of value in finding food only by coming into direct contact with it.

4. Decayed animal material is not attractive to these fish.

5. Although earthworm slime, human saliva and similar substances do attract Amiurus, the mucin which they contain is not a stimulating agent for the olfactory organs, though it may be so for the barbels.

6. All attempts to separate the proteins of earthworm, liver or blood resulted in preparations less stimulating than the original combination.

7. The fats from these materials were practically non-stimulating but the ether residues were decidedly stimulating.

8. It is suggested that the substances which stimulate olfaction in Amiurus are possibly of a protein nature since they are destroyed by putrefaction; they are not the products of protein decomposition; some of them are rendered ineffective by heat or addition of alcohol or acetone, others not; they are not of the nature of fats or etherial, volatile oils; and they are present in such small quantities that they are describable as "chemical traces," and can not be detected by the ordinary qualitative tests for proteins.

BIBLIOGRAPHY

DEAN, B.: '91. 19th Ann. Rept. New York State Comm. Fish., 299.
FORBES, S. A., AND R. E. RICHARDSON: '08. Nat. Hist. Survey of Illinois., iii.
HAMMARSTEN, O.: '14. A text-book of physiological chemistry. Trans. by J. A. Mandel. London.
HAWK, P. B.: '14. Practical physiological chemistry, Philadelphia.
HERRICK, C. J.: '02. Bull. U. S. Fish Comm., xxii, 237.
PARKER, G. H.: '08. Science, N. S., xxvii, 453.
'10. Journ. Exper. Zoöl., viii, 535.
'11. Journ. Exper. Zoöl., x, 1.
'12. Journ. Acad. Nat. Sci., Philadelphia, 2nd series, xv, 221.
'13. Bull. U. S. Bur. Fish., xxxiii, 63.
PARKER, G. H., AND R. E. SHELDON: '12. Bull. U. S. Bur. Fish., xxxii, 33.
PARKER, G. H., AND A. P. VAN HEUSEN: '17a. This Journal, xliv, 405.
'17b. This Journal, xliv, 463.
SHELDON, R. E.: '11. Journ. Exper. Zoöl., x, 51.
WODEHOUSE, R. P., AND J. M. D. OLMSTED: '17. Boston Med. Surg. Journ., clxxvii, 85.

THE RELATION OF THE DORSAL ROOTS OF THE SPINAL NERVES AND THE MESENCEPHALON TO THE CONTROL OF THE RESPIRATORY MOVEMENTS

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The general history of the work upon the nervous mechanism of respiration begins with the experiments of LeGallois and Flourens in the early part of the nineteenth century. In 1811 LeGallois (1) demonstrated that after transection below the medulla, all respiratory movements of the body cease with the exception of movements of the mouth, which cease also after section of the medulla. Flourens (2) amplified and confirmed this work (1842–1851). His experimental procedures began by removing the cerebrum, then the cerebellum, then the corpora quadrigemina of an animal (rabbits and pigeons were mostly used). Respiration persisted until sections of the medulla were made, then it failed. Reversing the operation and beginning with the lumbar spinal cord, making successive sections upward he found that

> In destroying the costal spinal cord, the rise and fall of the sides diminished gradually and when he had finished, had entirely disappeared.

As he continued to make sections upward, respiration was maintained, although with difficulty, by the diaphragm until the origin of the nerves of the diaphragm was reached when with their section and consequent cessation of the movements of the diaphragm, all effective respiration stopped, for the yawnings of the mouth and glottis which alone survived had no effect. He then proceeded in a reverse manner, removing the medulla by transverse sections from front to rear. The yawning movements disappeared first; then the dilation of the nostrils; the inspiratory movements of the trunk alone survived and finally these failed also. These experiments appear to indicate that the spinal respiratory nerves are unable of themselves to maintain rhythmic respiratory movements but are dependent on the action of a central coördinating mechanism situated somewhere above the lower end of the medulla oblongata.

Volkmann (1842), Longet (1847) and Schiff (1858) (3) showed that the central respiratory mechanism is a double organ which can be divided by a median longitudinal section without causing the death of the animal; and Longet, and more particularly Schiff, endeavored to show that this central mechanism is located in the nucleus of the grey matter in the alae cinereae in the lower part of the bulb, on each side beneath the floor of the fourth ventricle, and that the paths by which the impulse is conducted thence to the spinal cord run in the lateral bundles,

> Unilateral section of which at the lower level of the bulb, or at the level of the second or third cervical vertebrae, suffices to produce respiratory paralysis on the same side.

In opposition to this view, Brown-Sequard (4) enunciated his doctrine of "inhibitory centers." He showed in 1860 that if young animals were kept alive by artificial respiration for some time after section of the spinal cord below the medulla, when the artificial respiration was stopped, coördinated movements of the thorax and diaphragm might still be observed for a time. He therefore concluded that the center for respiration was not sharply localized in the medulla but extended throughout the spinal cord. The effects of section of the spinal cord below the medulla, he attributed to "inhibition" of these centers following the lesion of the cord, and he compared the phenomena with those of spinal shock.

This doctrine, including that of spinal respiration, was later presented in fuller form by Langendorff (1887) and Wertheimer (5) who observed that the respiratory muscles of the trunk could sometimes be made to contract after separation of the cord from the bulb in animals poisoned with strychnine, in animals with the cord artificially cooled or subjected to prolonged artificial respiration. Wertheimer declared that such contractions showed the power of the spinal cord to originate respiratory impulses.

Such an hypothesis has, however, been too often refuted to be at all acceptable at the present time. Schiff, in his early exposition of respiratory hemiplegia, demonstrated that section of the spinal cord at the level of the second and third cervical vertebrae paralyzes the respiratory mechanism.

Porter (6), in his study of the innervation of the diaphragm, followed a similar line of reasoning. He showed that since hemisections of the spinal cord above the phrenic nuclei do not inhibit the diaphragm

on the same side, it follows that two hemisections altogether separating the phrenic nuclei from the bulb do not inhibit the diaphragm on their respective sides. In other words, the arrest of thoracic and diaphragmatic breathing in consequence of the separation of the phrenic nuclei from the bulb is not an inhibition. But one explanation of the arrest is then possible; the phrenic nuclei effect no respiratory discharge after their separation from the bulb because they receive no impulses and cannot originate them. Hence the cells for the discharge of respiratory impulses are situated above the calamus scriptorius and not in the spinal cord.

In addition to this, Starling (7) has pointed out that cells from which the nerve fibers go to the respiratory muscles can, like the motor cells of other parts of the organism, be affected by impulses reaching them along various paths. Their normal activity in respiration depends upon impulses from the medulla but they can also be affected along other tracts derived ultimately from the posterior roots, at the same or higher levels of the cord.

Hering (8) has concluded that after division of all the dorsal roots of the frog, the motor cells cannot discharge when removed from peripheral stimulation, and in the case of respiration, he is convinced that

> The normal rhythm of respiration is bound up with the integrity of the accompanying centripetal nerves.

Of late years, however, the relative importance of the dorsal roots of the spinal nerves in the maintenance of respiration has been overlooked; perhaps the refutation of the ideas of "spinal respiratory centers" had a discouraging effect, certain it is that little or no mention of the dorsal roots and their connection with respiration is made in present-day literature. The best pronouncement with which I am familiar has been made by Luciani (9) who thus epitomizes their activity:

> When the auto-regulation by means of the vagi is suppressed, an abnormal type of respiratory rhythm appears which, although it provides for a degree of pulmonary ventilation sufficient to maintain life, must yet be termed dyspneic since it is not obtained without useless expenditure of energy. Under these conditions it seems to us probable that a self-regulation comes into play due to the rythmical and alternate excitation of the sensory paths of the inspiratory and expiratory muscles.

The question has often arisen as to whether there is a mechanism for the integration of the respiratory movements higher than the medulla. The opinions of the various authors who have written upon this subject appear to be somewhat divided.

Starling (7), in citing the work of Rosenthal and Marckwald, states,

> In the rabbit, section through the upper part of the medulla oblongata, separating the respiratory center from the higher parts of the brain, is equally with out effect on the depth and rhythm of the respiratory movements. A great change is observed, however, if the vagi are subsequently divided under these conditions.

Nikolaides (10) says that in rabbits, isolation of the medulla oblongata from above causes almost the same effect as double vagotomy.

Luciani (9) states that

> When the brain is extirpated to the level of a plane which passes along the inferior limit of the pons, or when the section is made at the level of this plane, it will be seen that after temporary disturbance, the animal continues to breathe in a regular, perfectly coördinated manner.

H. Newell Martin (11) found that

> On stimulation of the mid-brain of the rabbit, close to the iter and beneath the corpora quadrigemina, there is a respiratory regulating center similar to that of the corpora bigemina of the frog.

Marckwald (5) found that on blocking off the respiratory center from the brain above by the injection of paraffin into the common carotid, if these higher paths are cut off, the respiration remains regular, although deep, and perhaps in the course of time tends to resume its original type; but if the vagi are also sectioned, the respiration is entirely changed; periods of rapid breathing alternate with periods of complete cessation until the animal dies.

From the literature here quoted it will be seen that division of the vagi, in connection with section at the level of the corpora quadrigemina has been considered. The possible relationship of the dorsal roots of the spinal nerves has, however, been given no attention. I have therefore performed a series of experiments with a view to determining whether there is a possibility of such a relationship.

These experiments have extended over more than a year, and include results upon about forty cats. These animals were first etherized and then tracheotomized and ether was given by means of a tracheal cannula. Tracings of both costal and abdominal respiration were taken by means of Crile stethographs attached to Verdin recording tambours. I will consider the results first of section of the dorsal roots of the spinal nerves alone, then at the level of the posterior corpora quadrigemina alone and finally the effects of the two operations together.

After a control tracing of normal respiration (under ether) was taken, laminectomy was done and the dorsal roots of the spinal nerves were then sectioned, sometimes in both thoracic and cervical regions and sometimes in the cervical region only. If the dorsal spinal roots are cut in the thoracic region alone there is a diminution of costal respiration although abdominal respiration remains unaltered and the rate is very little changed; if the cervical dorsal roots also are involved, independent costal respiration disappears, such costal respiration as is present being passive and induced by the abdominal respiration as the tracing of March 2, 1918 (fig. 1) shows. Such respiration is slower than normal but the general character of the respiratory curve is not altered. When the dorsal roots are cut in the cervical region alone, thoracic respiration is not greatly changed. An animal whose dorsal spinal roots have been divided aseptically may be kept alive for an indefinite period. Such an operation, indeed, is analogous to the condition found in some cases of tabes dorsalis in which the functions necessary for the maintenance of life may be performed adequately enough although precision of movement is lacking.

It is of interest to observe in connection with the experimental work the remarkable compensatory power of the individual dorsal roots. If, for example, in sectioning the dorsal spinal roots in the thoracic and cervical regions, a single root on either side be left intact, costal respiration remains much better than the general severity of the operation and the number of roots cut would lead one to suppose possible. A study of the nervous system impresses one more and more with its remarkable adaptive facility in the rearrangement of channels for the conduction of nervous impulses when the normal ones are cut off, and this is particularly exemplified in the conduct of the dorsal spinal roots of the thoracic region of the cord.

Following is a protocol of an experiment in which the dorsal roots were divided.

March 2, 1918. Male cat (fig. 1).
Ether, tracheotomy.
Laminectomy.
Control tracing of respiration taken (part 1).
Dorsal spinal roots cut from third cervical to lower thoracic.
Respiration tracing taken (part 2).

In this experiment, the significant factor is the complete cessation of an active form of costal respiration, such slight passive movements as

are present being the results of the active diaphragmatic respiration. The rate, however, is not greatly altered.

It is evident that in the maintenance of respiration a central integrating mechanism is of first importance. We are well aware of the necessity of the integrity of the respiratory center in the medulla for the initiation of respiratory movements, but is there no mechanism for the integration of nervous impulses concerned with respiration higher than

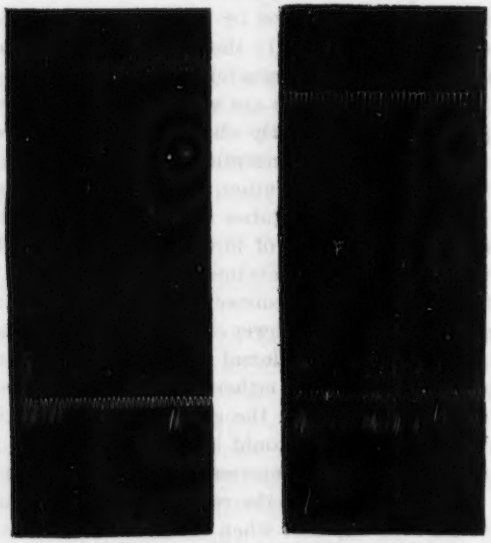

Fig. 1

Part 1. Respiration after laminectomy has been performed.

Part 2. Respiration after section of the dorsal roots of the spinal nerves from the third cervical to the lower thoracic. Upper tracing represents costal, lower abdominal respiration.

the medulla? In other words, if all portions of the brain above the medulla were removed, would respiration proceed in the same manner as before?

In the technique for the operation of section of the brain stem above the medulla, the carotids were first tied off to prevent excessive hemorrhage and the animal was then either decerebrated by removing the hemispheres from the cranial cavity or a trephine opening was made

over the occipital ridge and the corpora quadrigemina were sectioned through it.

I have found that sections in front of the corpora quadrigemina and between the anterior and posterior corpora produce no effect upon respiration; when, however, the section cuts into or behind the posterior corpora quadrigemina, there is a change in the character of the respiration. It appears to become slower and less regular than the normal type; still, it is hardly of a gasping character and maintains a very fair type of ventilation.

The difference in conduct between the results of this operation and that of complete section of the dorsal roots in the thoracic and cervical regions is one of degree rather than kind. In the latter case, a few channels for sensory impulses may remain above and below the sections—and we have mentioned the compensatory power of the dorsal roots in this respect—while the conduct in the former case implies a total lack of these sensory impulses.

In this connection, we cite the protocol of

March 16, 1918. Male cat (fig. 2).
Ether, tracheotomy.
2.00 p.m. Normal respiration (part 1).
2.45 p.m. Carotids tied off, then section behind corpora quadrigemina.
3.30 p.m. Good respiration, see tracing (part 2).

Such respiration as this gives no indication of the dyspnea which some authors have found and does, to some extent, resemble the slowing obtained after double vagotomy. I have observed at various times, however, that if after section is made there occurs a hemorrhage into the fourth ventricle, which causes a clot producing pressure upon its floor, then dyspnea always occurs. But if no such hemorrhage occurs, dyspnea is not present except in a very slight degree.

As I have previously indicated, the spinal cord has not been regarded as an important factor in respiration, during late years, and even when the possibility of spinal respiratory centers was under consideration few authors ever expressed the idea of a relationship between the dorsal roots and these spinal centers. It has been shown in a previous paper (15) that the dorsal roots undoubtedly play an important part in the sensory mechanism of costal respiration and that fibers concerned with afferent impulses pass up the spinal cord; our present work has served to confirm these findings and to extend them. Moreover, section of the brain stem at the level of the posterior corpora quadrigemina produces

immediate and lasting effects upon the respiration. Since the mesencephalon contains afferent and efferent fibers from the spinal cord, the question presents itself as to possible relationships between the dorsal roots of the intercostals and the mesencephalon as shown by the effect of section at the level of the posterior corpora quadrigemina and the dorsal roots of the spinal nerves in the cervical-thoracic region. Following is a protocol of such an experiment.

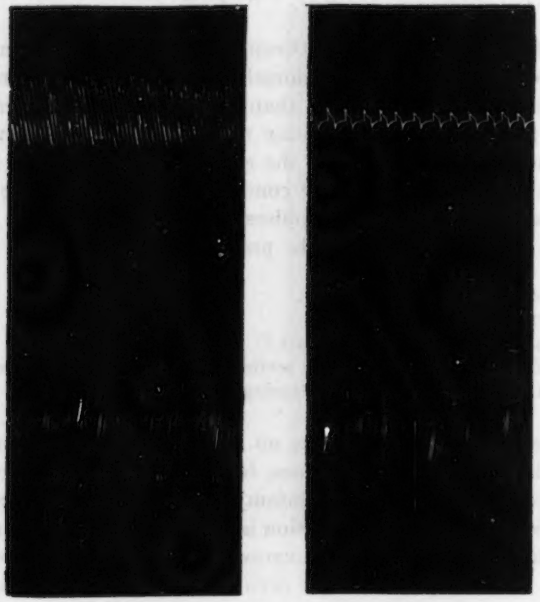

Fig. 2

Part 1. Normal respiration.
Part 2. Respiration after section behind the posterior corpora quadrigemina. Upper tracing represents costal, lower abdominal respiration.

July 13, 1917. Female cat (fig. 3).
Ether, tracheotomy, laminectomy (part 1).
2.40 p.m. Carotids tied off.
2.45 p.m. Decerebration.
2.55 p.m. Section behind the corpora quadrigemina (part 2).
3.20 p.m. Dorsal roots in cervical and upper thoracic regions cut (part 3).

From this experiment an interesting phenomenon may be observed, namely, that *after section of the posterior corpora quadrigemina, subsequent section of the dorsal roots is followed by no additional effects.* Such a finding leads one to conclude that certain of the sensory impulses at least, if not all connected with respiration from the dorsal roots of the

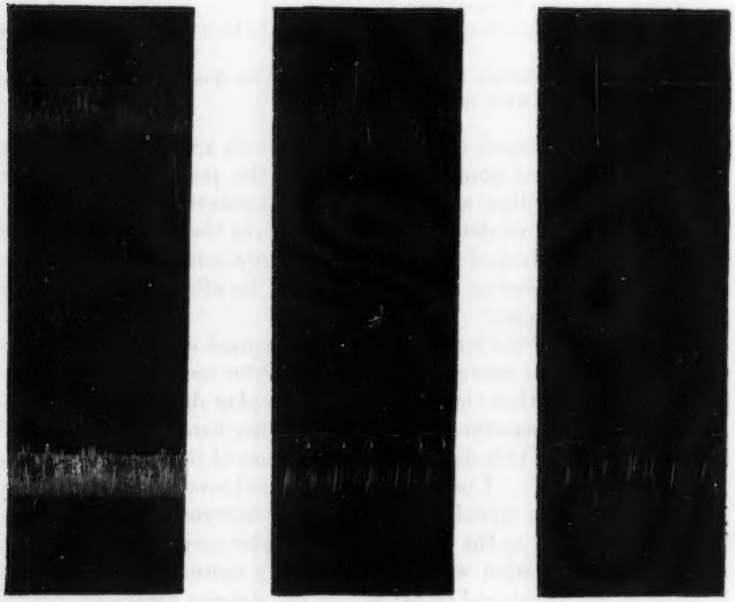

Fig. 3

Part 1. Normal respiration.
Part 2. Respiration after section behind the posterior corpora quadrigemina
Part 3. Respiration after section of the dorsal spinal roots in the cervica and upper thoracic regions. Upper tracing represents costal, lower abdomina respiration.

intercostals, pass through the posterior corpora quadrigemina since the difference in severity between the results of the two operations is about the difference that might be expected between total and partial elimination of the afferent impulses from the dorsal roots. Moreover, the fact that section of the dorsal roots after section of the corpora quadrigemina produces no change in the character of respiration shows that the entire effect was obtained by the first division.

Section of the dorsal roots before the corpora quadrigemina are sectioned does leave some additional effect to be gained by the latter operation, as the following protocol shows.

March 5, 1918. Male cat (fig. 4).
Ether, tracheotomy, laminectomy.
2.45 p.m. Control tracing (part 1).
3.05 p.m. After section of the dorsal roots in the thoracic and lower cervical regions (part 2).
3.30 p.m. After section of the posterior corpora quadrigemina. Note the Cheyne-Stokes respiration (part 3).

From this experiment it is evident that there are still some afferent intercostal impulses going through until the posterior corpora are divided—not until then are all intercostal impulses cut off.

Such corroborative detail points strongly to the probability of the existence at the level of the posterior corpora quadrigemina of some station closely related to the integration of the afferent impulses from the respiratory "cage."

While certain of the motor impulses concerned in the skilled movements of respiration must originate in the motor areas of the cerebrum it is hardly likely that these are called into play during normal respiration or during anaesthesia; and on the other hand, a purely medullary type of respiration due to the movements of the diaphragm alone is not normal either. I believe, therefore, that sensory fibers from the dorsal roots of the spinal nerves from the intercostals travel up the brain stem as high as the level of the posterior corpora quadrigemina, where some connection with the descending motor fibers is effected. In other words, the dorsal spinal nerves and a region for the integration of respiratory impulses at the level of the posterior corpora belong to the same system. The fact that vagi and mesencephalon are unrelated in this manner is what makes section of the vagi in this connection so much more fatal than section of the dorsal roots—a relation which will be discussed in a subsequent paper.

The time element concerned in section of the dorsal roots and the corpora quadrigemina may also be considered. It is well known from clinical evidence that people in whom accident or disease has destroyed the dorsal spinal nerve roots are able to support life very adequately. Stewart (12) has cited the case of a man in whom all the ribs became completely immovable from disease of the spine in the lower cervical region. He was able to lead an active life and carry on his business although he breathed entirely by means of the diaphragm and abdom-

inal muscles. Whether an animal in which both the dorsal roots and the corpora quadrigemina were destroyed could maintain life very long, I am not prepared to state; but experimentally, under anaesthesia, good respiration may be maintained for several hours subsequent to these operations.

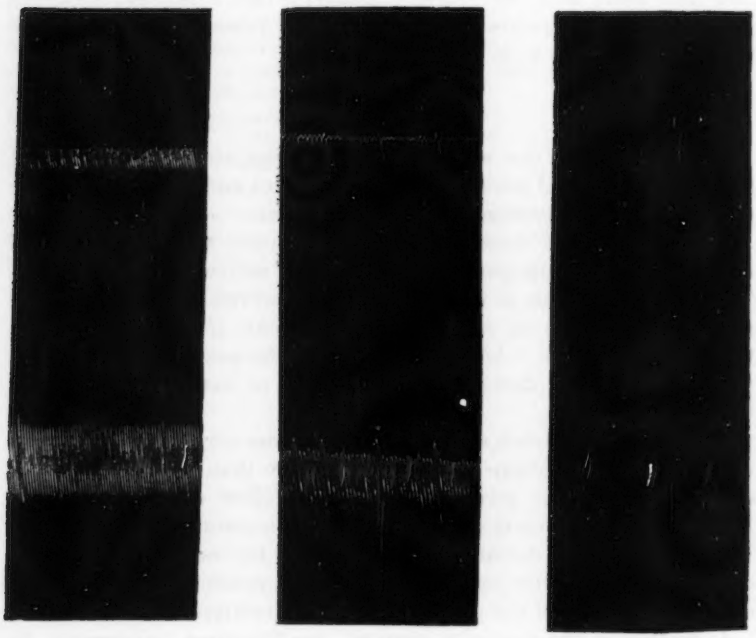

Fig. 4

Part 1. Respiration after laminectomy.

Part 2. Respiration after section of the dorsal roots in the thoracic and lower cervical regions.

Part 3. Respiration after section behind the posterior corpora quadrigemina. Note the Cheyne-Stokes respiration. Upper tracing represents costal, lower abdominal respiration.

Sherrington (13), in his work on decerebrate rigidity, describes the persistent tonic spasm which occurs in certain groups of muscles after section of the brain stem in front of the corpora quadrigemina. The groups of muscles which are contracted are the retractors of the head

and neck, the muscles of the tail, the extensors of the elbow, knee, shoulder and ankle—the antigravity muscles. The spasm depends on the integrity of the dorsal spinal roots and appears not at all, or only imperfectly, in the limbs of which the corresponding dorsal nerve roots are divided. Section at that level of the corpora quadrigemina also does away with decerebrate rigidity, a fact which offers further confirmation that certain fibers of the dorsal spinal nerve roots have end stations at this level.

CONCLUSIONS

In summarizing the effects upon the respiratory movements of section of the dorsal roots of the spinal nerves and at the level of the posterior corpora quadrigemina, my findings are:

1. Section of the dorsal roots of the thoracic and cervical spinal nerves results in a diminution or cessation of active costal respiration. The effect of section of both thoracic and cervical nerves is a more marked diminution of costal respiration than after section of the thoracic roots alone. After section of the thoracic roots, abdominal respiration remains unchanged and there is no marked alteration in the respiratory rate.

2. Section of the brain stem below the anterior corpora quadrigemina results in a slower, deeper form of respiration than normal somewhat similar to the most severe effects which follow double vagotomy. Abdominal respiration is more prominent than costal.

3. Section of the dorsal roots of the spinal nerves after section into or behind the posterior corpora quadrigemina produces no more severe effect than section of the posterior corpora quadrigemina alone.

4. Section of the posterior corpora quadrigemina subsequent to section of the dorsal roots of the spinal nerves produces an effect on respiration somewhat greater than when the dorsal roots alone are sectioned.

5. The general relationship of afferent to efferent spinal nerve roots which Sherrington (14) describes obtains also in the afferent and efferent intercostal roots.

I wish to express my thanks to Professor F. H. Pike of this department for his valuable suggestions and criticisms of this work.

BIBLIOGRAPHY

(1) LeGallois: Experiments sur la principe de la vie, 160, 1824.
(2) Flourens: Recherches experimentales sur les propriecées et les fonctions du systéme nerveux, 2d ed., 173, 1848.
(3) Rosenthal: Hermann's Handb. Physiol., iv, Leipzig, 1882.
(4) Brown-Sequard: Journ. d. Physiol., 1858, i.
(5) Marckwald: Zeitschr. f. Biol., 1887.
(6) Porter: Journ. Physiol., xvii, 455.
(7) Starling: Schäfer's Textbook of physiology, ii, 288.
(8) Hering: Arch. f. Physiol., 1893, lviii, 614.
(9) Luciani: Human physiology, i.
(10) Nikolaides: Arch. f. Physiol., 1905, 465.
(11) Martin: Journ. Physiol., i, 370.
(12) Stewart: Manual of physiology, 6th ed., 223.
(13) Sherrington: The integrative action of the nervous system.
(14) Sherrington: Schäfer's Textbook of physiology, ii, 802.
(15) Pike and Coombs: This Journal, 1917, xlix, 395.

VII. THE EFFECT OF ADRENALIN ON THE IRRITABILITY AND CONTRACTILITY OF MAMMALIAN NERVE-MUSCLE PREPARATIONS AFTER DEATH

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That the reaction of a muscle after fatigue is acid to litmus has been known for over half a century. Du Bois Reymond (1) in 1859 demonstrated that muscles become acid upon stimulation. Professor Schwann wrote in a letter to Du Bois Reymond of chasing a chicken until it was completely exhausted, after which he killed it and found a distinct acid reaction in the muscles. During the same year Funke (2) made note of this reaction. Four years later Ranke (3) was able to demonstrate that this acid reaction was caused by a production of lactic acid, and in 1865 he proved definitely that paralactic acid, monopotassiumphosphate and carbon dioxide are the products given off during the process of fatigue and that if these substances are injected into the circulation or irrigated through the muscles of unfatigued animals, typical fatigue results are obtained. Heidenhain (4), Landau and Pacully (5), Marcuse (6), Gleiss (7), Landsberger (8), Boehm (9), Osborne (10), Fletcher and Hopkins (11) and Fletcher (12) have confirmed Ranke's results. Lee (13), Burridge (14) and Schenck (15) were able to reproduce the fatigue curve by irrigating muscles with the above products. Geppert and Zuntz (16) have shown that the alkalinity of the blood is diminished after muscular exercise.

A similar acid reaction is always found in a muscle approaching a state of rigor mortis. Here as in fatigued muscles the reaction is the result of the accumulation of lactic acid (Du Bois Reymond (1), Ranke (3), Boehm (9), Osborne (10), Fletcher and Hopkins (11) and Fletcher (12)). Fletcher and Hopkins found that in frogs most of the lactic acid is produced while the muscle is still flaccid and irritable, before rigor has been reached. Fletcher noticed that in mammals the accu-

mulation of lactic acid takes place most rapidly during the first three hours after death (0.453 per cent at the end of three hours and 0.513 per cent at the end of nine hours, extracted in the form of zinc lactate).

This series of experiments was made in order to determine, since the chemical and physiological changes in the two conditions are the same, whether adrenalin, which has a marked bettering effect on fatigued muscles (17), might not also affect muscles approaching rigor.

METHOD

The operative procedure and the apparatus used here were the same as those employed by one of us in a previous work (17). Cats were used. The tibialis anticus muscles were prepared for perfusion and at the moment that the cannulas were inserted into the vessels the animals were killed with ether. The nerve was exposed and placed in a Sherrington shielded electrode. The tendon of the muscle was fastened to a muscle lever and stimulated ninety times per minute. The medium for irrigation was a warm (37.5°C.) Ringer's solution, at a pressure of 70 cm. of water, containing only the oxygen absorbed from the air.

RESULTS

It was observed in these experiments that muscles lost their irritability to electrical stimuli with varying rapidity. Some muscles showed clearly at the end of an hour the effect of diminished supply of oxygen by the decreased irritability to the electrical stimulus and a decreased height of contraction. Others continued to respond almost normally at the end of that time.

Figure 1 was taken from an animal which had been dead for 1 hour and 6 minutes. Perfusion was begun 17 minutes before the beginning of stimulation. The muscle was able to do only one-third as much work as the corresponding muscle of the other limb had done, under similar operative conditions, immediately after the animal had been killed. The muscle in figure 1 contracted for 2.6 minutes, the contractions reaching at the highest point 2.7 cm., after which they dropped gradually to 0.5 cm., at which point, *1*, adrenalin (1 cc. of 1:100,000 solution) was injected into the perfusion fluid near the arterial cannula. This prolonged muscular contraction for 1 minute. At *2*, muscular contraction having almost ceased, adrenalin (0.5 cc. of a 1:1000 solution) was injected. Immediately there was produced a marked vasoconstriction and simultaneously an increase in the height of muscular

contraction, at its highest point 29 fold. The effect of this injection lasted more than 7 minutes. This increase in height of muscular contraction is 11 per cent higher than the first response of the muscle recorded here. Adrenalin therefore not only counteracted the effect produced by fatigue but also a part of the decreased efficiency of the muscle caused by the lack of blood supply and the consequent death changes.

During this research it was repeatedly noticed that the threshold stimulus markedly increases during death changes. In some animals

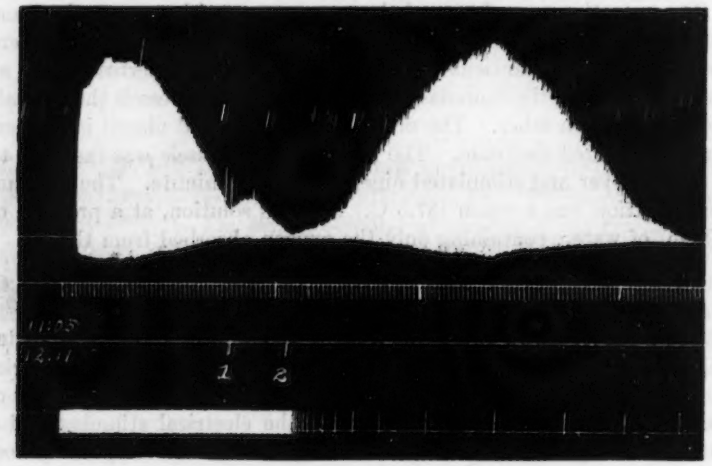

Fig. 1. In this and the following figures the upper curve is the record of muscular contractions, below it the time interval. The lowest record indicates the rate of flow of the perfusion fluid through the muscle. *1*, adrenalin 1 cc. (1:100,000); *2*, adrenalin 0.5 cc. (1:1000). Time in 5 seconds.

the muscle failed to respond to electrical stimulation of its nerve after the circulation had been removed for one hour. In others the threshold stimulus was increased five to one hundred times above normal. In all cases where any response could be obtained adrenalin lowered the threshold stimulus. An example may be cited here. In one animal in which the circulation was removed for 1 hour and 47 minutes, the current strength necessary to arouse the muscle into activity upon electrical excitation through its nerve was 28 Z units (18). In the opposite limb the corresponding muscle at death had responded to a

stimulus of 2 Z units. After an injection of adrenalin (1 cc. of a 1:100,000 solution) the threshold of 28 Z units was decreased to 10 Z units. Another injection of adrenalin (0.5 cc. of a 1:1000 solution) lowered it to 6 Z units. This demonstrates that the action of adrenalin in muscles undergoing death changes is similar to that observed in fatigued muscles.

Figure 2 is a record obtained from a kitten weighing 1.5 kilos which received an intravenous injection of 15 mgm. of hirudin as an anticoagulant. The animal was killed at 2:32 and the perfusion was begun at 3:08 (36 minutes later). At 3:15 or 42 minutes after the animal was killed and 6 minutes after perfusion was started stimulation of the muscle at the rate of ninety times per minute was begun. The muscle responded only slightly to a strength of stimulus of 485 Z units. (See *x*) At *1* and *2* injections of adrenalin chloride (2 cc. of 1:100,000 solu-

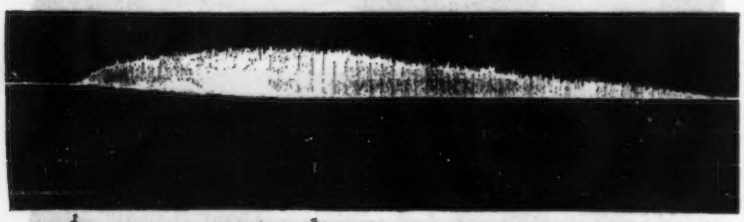

Fig. 2. Adrenalin 2 cc. (1:100,000) injected into the perfusion fluid at *1* and *2*. Both make and break shock contractions present. Time in 30 second intervals. Reduced to $\frac{2}{3}$ original size.

tion) were made into the perfusion fluid. There was, as a result of the first injection, approximately a 14 fold increase in the height of muscular contraction and as a result of the two injections the betterment in muscular contraction lasted for more than 5 minutes.

Figure 3 is presented to show the effect of adrenalin on nerve-muscles in which the irritability was completely lost even to strong electrical currents. The animal from which this record was made had been dead for 1 hour and 5 minutes. In the beginning of the experiment the strength of the current was not known but the pointer of the secondary coil stood at 0, indicating a current of more than 799 Z units. The muscle was perfused for 12 minutes before excitation was begun. At *1*, indicated in the record, adrenalin (2 cc. of a 1:100,000 solution) was injected in the usual manner and in less than 1 minute the muscle responded vigorously to both make and break shocks. To eliminate

the make shock contraction, the current was decreased to 220 Z units at *12* but due to the adrenalin the irritability of the nerve-muscle gradually increased so that the current had to be decreased to 59 Z units at *15* before there was a permanent loss of the make shock contraction. The height of contraction increased from 0 to 1.8 cm. as a result of the injection. At *2* another 2 cc. of adrenalin (1:100,000) was injected which brought about a betterment of more than 60 per cent. In the corresponding muscle of the opposite limb, from which the circulation had been cut off for 2 hours, three injections of 2 cc. each of adrenalin (1: 100,000) were necessary before the muscle could be made to contract.

This recovery of contractility and irritability after total loss could not be brought about by adrenalin in every case. It was observed that the muscles of animals dead for three or four hours could not be

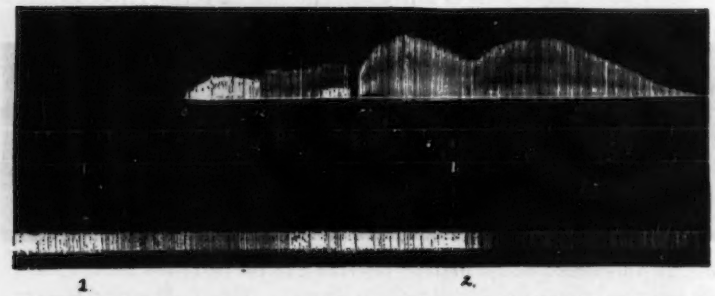

Fig. 3. Explained in text. *1* and *2*, adrenalin 2 cc. (1: 100,000). Time in 30 seconds. Reduced to ⅔ original size.

restored to activity by adrenalin upon excitation of their nerves. It was noted, however, that in some of these cases adrenalin was capable of restoring the muscle fibers to activity by direct excitation of the muscle, individual fibers responding by twitching.

DISCUSSION

Adrenalin affects muscles undergoing death changes as it does fatigued muscles. It increases muscular activity (the height of muscular contraction) and increases the irritability of the nerve-muscle to electrical excitation (decreasing the threshold stimulus).

It probably acts upon the same substance in these muscles as in fatigued muscles. Here as in fatigued muscles there are three possible points of action:

a. It may assist or hasten the conversion of glycogen into available sugar to be used as energy. Since sugar is used in muscular contraction it is conceivable that any change which would hasten its production would better the height of muscular contraction. In muscles undergoing death changes much of the glycogen has been changed into lactic acid. A considerable quantity, however, probably remains from one to three hours after death to be converted into sugar, which conversion may be assisted by adrenalin.

b. Adrenalin may hasten the reconversion of lactic acid into sugar so that more available energy is present. (Transformation of fatigue products.)

c. The oxidation of lactic acid into carbon dioxide and water may take place more rapidly with the aid of adrenalin, a toxic substance thus being changed into less harmful substances. (Destruction of fatigue products.)

SUMMARY

Adrenalin has the same action upon nerve-muscle preparations undergoing death changes as it has upon fatigued muscles. It decreases the increased threshold stimulus and betters the height of muscular contraction.

BIBLIOGRAPHY

(1) Du Bois Reymond: Arch. f. Anat., 1859, 849.
(2) Funke: Müller's Arch. f. Anat. u. Physiol., 1859, 835.
(3) Ranke: Arch. f. Physiol., 1863, 422; Ibid., 1864, 320. Tetanus, Leipzig, 1865.
(4) Heidenhain: Studien der Physiologie Institute zu Breslau, 1863, iv, 250.
(5) Laudau and Pacully: Arch. f. d. gesammt. Physiol., 1869, ii, 423.
(6) Marcuse: Arch. f. d. gesammt. Physiol., 1886, xxxix, 426.
(7) Gleiss: Arch. f. d. gesammt. Physiol., 1887, xli, 69.
(8) Landsberger: Arch. f. d. gesammt. Physiol., 1891, L, 339.
(9) Boehm: Pflüger's Arch., 1880, xxiii, 44.
(10) Osborne: Journ. Physiol., 1901, xxvi, 49.
(11) Fletcher and Hopkins: Journ. Physiol., 1906, xxxv, 247.
(12) Fletcher: Journ. Physiol., 1913, xlvii, 361.
(13) Lee: Journ. Amer. Med. Assoc., 1905, xlv, 1776; Ibid., 1906, xlvi, 1492; Centralbl. f. Physiol., 1906, xx, 869.
(14) Burridge: Journ. Physiol., 1911, xli, 285.
(15) Schenck: Arch. f. d. gesammt. Physiol., 1891, L, 117.
(16) Geppert and Zuntz: Arch. f. d. gesammt. Physiol., 1895, lxii, 295.
(17) Gruber: This Journal, 1913, xxxii, 438; Ibid., 1914, xxxiii, 335; Ibid., 1917, xliii, 530.
(18) Martin: Measurement of induction shocks, New York, 1912.

A NOTE ON THE SUPPOSED RELATION OF THE SYMPATHETIC NERVES TO DECEREBRATE RIGIDITY, MUSCLE TONE AND TENDON REFLEXES

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The question of the sympathetic innervation of striated muscle is still far from settled. As the evidence accumulates, the probability diminishes that a simple explanation of tonus has at last been found. For a time the researches of Boeke in anatomy, and of the physiologist here quoted, had made it seem probable that tonic muscular contraction was due to sympathetic innervation. In 1913 Dusser de Barenne (1) tried the effect of unilateral section of the abdominal sympathetic chain in decerebrate cats, and found that in only five out of nine such preparations was there an ipsilateral diminution of the rigidity. He concluded that the tonic impulses causing decerebrate rigidity did not reach the muscles of the hind leg by way of the sympathetic. The next year Langelaan (2), writing on tonus, stated that decerebrate rigidity was to be regarded as a spasm of sympathetic origin. In 1915 deBoer (3) published a monograph on tonic innervation, summarizing his work of several years, and gave evidence to show that extirpating the abdominal sympathetic chain on one side in cats caused ipsilateral hypotonicity with exaggeration of the tendon reflexes. The loss of tone showed in the affected hind leg by its diminished resistance to passive flexion and by the fact that when the cat was held up by the skin of its neck, this limb hung lower than the contralateral one, and showed greater extension at the joints. The animal's tail was observed to hang toward the unaffected side.

In 1916 these experiments of deBoer and those of Dusser de Barenne were modified and repeated and the results of the work on mammals are here published. A series of experiments was also done on frogs to test deBoer's observation that cutting the rami communicantes of

the abdominal sympathetic in frogs causes a loss of tone in the ipsilateral leg muscles. Sixty-one frogs were operated on in different ways and although the simple cutting of the rami usually seemed to cause the leg to hang lower, no consistently corroborative evidence was obtained from stimulation or degeneration experiments. These results are considered too equivocal to be worth publishing at length.

More recently Van Rijnberk (4) has repeated the experiments of Dusser de Barenne and finds that in no cases does the section of the abdominal sympathetic affect the development of the decerebrate rigidity. Dusser de Barenne (5), however, has repeated deBoer's experiment and has found a lessening of muscular tone in the corresponding hind leg after cutting the abdominal sympathetic chain.

In my mammalian experiments the abdominal sympathetic was cut before decerebration in five cases and afterwards in one case. The effect of stimulating the sympathetic chain was tried out, also the effect of inhibiting decerebrate rigidity by cerebellar stimulation[1] with and without an intact sympathetic chain. Besides this, repeated observations were made on six cats, after their recovery from the sympathetic excision, to see if the muscular tonicity or tendon reflexes had been affected.

CONDENSED PROTOCOLS

Cat 1. February 9, 1916. Operated under ether and aseptic precautions; flank incision and extraperitoneal excision of 4th and 5th lumbar ganglia on the right.

February 10. No evidence of hypotonia of right leg or tail.

February 11. No evidence of hypotonia of right leg or tail.

Abdominal operation, with excision of 6th and 7th lumbar ganglia and 1st sacral ganglion, on the right.

February 15. No evidence of hypotonia of right hind leg; it is somewhat more stiffly held than the left. Tail carried evenly. Operation for decerebration: transection just anterior to superior colliculi; strong rigidity immediately, equal on the two sides. Four hours later rigidity still present and equal bilaterally. Cat killed. Autopsy substantiates operation.

Cat. 2. February 19, 1916. Extraperitoneal excision of 6th left lumbar sympathetic ganglion.

February 20. No evidence of hypotonia.

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[1] In a previous paper (6) the writer has described how stimulation of the anterior lobe of the cerebellum on either side, or direct stimulation of the underlying superior cerebellar peduncle, causes ipsilateral inhibition of the extensor rigidity in decerebrate cats.

February 21. Tail hangs to right more than to left, but not definitely. Legs show no difference. Decerebrated: immediate rigidity, equal on the two sides, tail stiff and in midline; observed for one hour. Autopsy shows 6th left lumbar ganglion absent.

Cat 3. February 23, 1916. Operation; piece of abdominal sympathetic 1.5 cm. long excised with 5th right lumbar ganglion.

February 24 to March 17. Cat kept alive and observed for twenty-three days; tail always hung in midline. With 50 gram weight attached to each hind leg and the cat held up by the neck, the legs hang at varying heights; on four days the right seemed definitely lower, on two days the left, and on four days there was no difference. No increase in knee jerks on either side; no loss of resistance to passive motion found in either hind leg.

March 17. Decerebration: rigidity develops after five minutes, more on right than left, later equal on two sides, tail stiff in midline. Stimulation of anterior lobe of cerebellum to right and left of midline gives ipsilateral inhibition of the extensor rigidity. Autopsy shows 5th right lumbar ganglion of sympathetic chain to be lacking; the nerve trunk is divided and embedded in scar tissue.

Cat 4. February 29, 1916. Extraperitoneal operation; left abdominal sympathetic chain cut between the 4th and 5th lumbar ganglia.

March 1. With 50 gram weights attached to legs, the left usually hangs lower than the right.

March 4, 9 and 10. Tail hangs in midline, no difference in tonus of the legs either on passive motion or on holding animal up with weights on legs. No difference in knee jerks.

March 10. Decerebration: slight rigidity for three hours, equal on the two sides. Autopsy checks up operation.

Cat 5. March 6, 1916. (Decerebration before cutting sympathetic.) 11.45: Decerebration: rigidity comes on quickly, equal on two sides.

12.30 Exposure of right sympathetic chain, loose ligature laid around it.

1.50: Right Achilles tendon attached to lever recording on drum. Rigidity medium throughout this time.

2.20 to 3.30: Tetanic induction shocks to the sympathetic nerve at the ligature (between 4th and 5th lumbar ganglia) cause no increase in the tonus of the right gastrocnemius recording on the drum.

3.40: During good rigidity, the sympathetic is cut at the ligature and no relaxation of the right gastrocnemius occurs. After this, rigidity remains equal on both sides in the legs and tail.

4.30: Cerebellum exposed and electrical stimulation of the anterior lobe in the midline causes inhibition of the rigidity, as is shown by relaxation of the right gastrocnemius.

Cat 6. March 10, 1916. Abdominal operation; sympathetic chain exposed and the 6th and 7th right lumbar ganglia avulsed with a portion of the nerve.

March 13. Tail hangs slightly to left; knee jerks greater on left; legs hang equally when weighted.

March 15. Tail hangs slightly to right; knee jerks constantly greater on right; less resistance to passive motion on right; left leg hangs lower with 50 gram weights.

March 16. Decerebrated: rigidity comes on in five minutes; moderate intensity, equal bilaterally. Each Achilles tendon attached to a lever registering on drum. Cerebellum exposed and effect of stimuli registered; preparation continues active with rigidity for four hours. Tetanic induction shocks to anterior lobe of cerebellum cause inhibition of rigidity in both hind legs, as in cats with intact sympathetic nerves (6). Autopsy checks up operation; bronchopneumonia present.

Cat 7. March 14, 1916. Abdominal operation; dissected out 3d, 4th and 5th right lumbar ganglia with intervening sympathetic nerve.

March 15. Tail hangs slightly to left; knee jerks greater on left; less resistance to passive motion on right; with 50 gram weights left leg hangs lower than right.

March 16. Animal died; peritonitis; autopsy checks up operation.

SUMMARY

Examination of the above protocols shows that in five cats in which unilateral division of the abdominal sympathetic chain had been previously performed (three to twenty-three days before decerebration) the development of decerebrate rigidity in the hind legs was unaffected by this operation. In three cases the anterior lobe of the cerebellum was exposed and stimulation with induction shocks was tried. In all three inhibition of the rigidity was recorded as in normal animals (6).

In six cats observations were repeatedly made of the knee jerks, the tonus of the hind legs and the tonus of the tail. Previous investigators have described hypotonia of the leg on the side of the operation; this lack of tone was said to have appeared when the animal was lifted by the neck—the more flaccid leg hanging with its foot lower than that of the more tonic, equal weights being attached to the legs to bring out the phenomenon. No such ipsilateral hypotonia was found in these experiments nor was there any constant change in the knee jerks. Passive flexion of the hind legs was tried repeatedly and no constant difference could be detected between the operated and unoperated sides. It was noticed, however, that slight differences in grasping and holding the cat's neck caused changes in the hanging of the legs and in their stiffness; there seemed to be a synergic relation between the position of the neck and the tonus of the hind legs (7), which might explain some of the former observations.

Hypotonia of the tail, as described by deBoer, shows by an asymmetrical posture of the tail. It was described as being held toward the side on which the sympathetic was intact, the theory being that the muscles of this side of the tail had the greater tonus. In six of

the cats of the present series the position of the tail after operation was noted. In two it seemed to hang slightly toward the intact side, suggesting an ipsilateral hypotonia; in one it consistently deviated toward the operated side and in three there was no deviation from the normal midline position.

In one case the decerebration operation was performed first and later the sympathetic was exposed, stimulated and finally cut while the tonically contracted gastrocnemius was registering on a revolving drum. No change in the steady contraction was recorded.

CONCLUSIONS

Section of the abdominal sympathetic chain in cats:

1. Has no effect on decerebrate rigidity, either by preventing its development or its inhibition.
2. Causes no obvious hypotonicity of the hind legs or tail.
3. Causes no change in the tendon reflexes.

Stimulation of the abdominal sympathetic chain causes no tonic contraction of the ipsilateral hind leg.

BIBLIOGRAPHY

(1) Dusser de Barrenne: Folia neuro-biol., 1913, vii, 651.
(2) Langelaan: Neurol. Centralbl., 1914, xxxiii, 1140.
(3) deBoer: Zeitschr. f. Biol., 1915, lxv, 239.
(4) Van Rijnberk: Arch. neerl. de physiol., 1917, i, 702.
(5) Dusser de Barenne: Pflüger's Arch., 1916, clxvi, 145.
(6) Cobb, Bailey and Holtz: This Journal, 1917, xliv, 239.
(7) Dusser de Barenne: Folia neuro-biol., 1914, viii, 413.

THE NON-EFFECT OF CORPUS LUTEUM PREPARATIONS ON THE OVULATION CYCLE OF THE RAT

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The first and one of the most attractive of the many theories as to the function of the corpus luteum of the mammalian ovary is that suggested by Beard (1) and reiterated by Prenant (2), that this gland may by an internal secretion inhibit ovulation during pregnancy in order to spare the organism for a time the stresses of the ovarian cycle which, as Beard supposed, by finally asserting themselves bring about parturition; if the ovulation cycle reappears prematurely, abortion occurs and hence the necessity of some such inhibitory mechanism as the corpus luteum was supposed to furnish.

Though Beard's theory as a whole did not retain general interest, that part of it which refers to the corpus luteum rests upon the undoubted fact that there is a pause in ovulation between the regular periods and during pregnancy, and hence the possibility of an inhibitory function of the corpus luteum is not to be neglected.

Among the mass of speculative discussions of this subject there have been two important efforts to test the hypothesis by experiment. Leo Loeb (3) attempted to determine the normal period of ovulation in guinea pigs and then to remove the corpora lutea by operation and to determine the time of the ensuing ovulation. As a result of his experiments he believed the period between two ovulations to be variable but usually from twenty to twenty-five days, and never less than fifteen days. If the corpora lutea are removed, the total interval between the ovulation previous to operation and that next following is from twelve to sixteen days, usually about fourteen. The occurrence of ovulation was determined by killing the animals at varying times of the cycle and studying the ovaries. Recently Stockard and Papanicolaou (4) have introduced a much more exact method of following the reproductive cycle in living animals by the observation of vaginal changes, and have determined the ovulation cycle of the guinea pig to

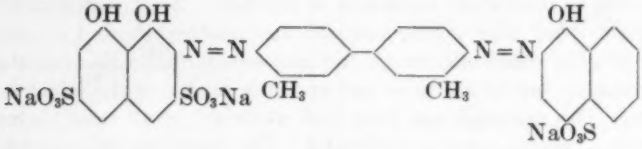
vary from fifteen to seventeen days, averaging fifteen and one-half. Thus it would seem that Loeb's experiments do not prove an acceleration of ovulation following the removal of the corpora lutea.

Pearl and Surface (5) have adopted the ingenious method of administering corpus luteum substance to hens, in which the function of ovulation is more frequent and much more easily measured than in mammals. In a small series of carefully guarded experiments, using hens which were laying regularly, the intra-abdominal injection of the commercial desiccated corpus luteum substance prepared by Armour & Company, in doses of 0.4 to 0.5 gram per kilogram of body weight, suspended in normal saline solution, produced in most cases an inhibition of ovulation lasting for several days, and in all cases the number of eggs laid in the ten days following the injection was markedly less than in the same period before treatment. The administration of normal saline alone and of boiled corpus luteum substance, did not produce inhibition of ovulation.

It has seemed to the present writers that an application of the same experiment to mammals would be worth an attempt, in spite of the great difficulties attending the determination of ovulation in the viviparous animals. We need first an animal in which ovulation is known to be frequent and fairly regular, and second, a sure means of detecting the occurrence of ovulation during the period of experiment. Our work was concluded before we could make use of Stockard's observations, and meanwhile we had developed an experimental method which gives an exact count of the number of follicles which have ruptured during a given period, by direct inspection of the ovaries and without the uncertainties formerly occurring. This method we owe to the generous advice of Professor Evans and Dr. J. A. Long of this University, who put freely at our disposal the results of their long studies upon vital staining of the corpus luteum and of the ovarian cycle of the adult rat, Mus norvegicus.

The studies of Drs. Long and Evans, now in course of preparation for publication, show that the ovulation cycle of the rat varies from four to eleven days but may be fairly constant for any one rat. Ovulation always occurs about eighteen hours after the birth of a litter, and we can make this a convenient starting-point for following the cycle by administering a vital stain which will color all corpora lutea existing in the ovary. (In this animal the corpora persist for many weeks, so that several successive crops are present at one time.) Follicles rupturing subsequently to the time of staining will give rise to unstained corpora lutea.

The dye furnished us by Doctor Evans was a salt-free sample of a benzidine compound of blue color having the following formula:



His tests had shown that this dye, when administered subcutaneously or intra-abdominally, is readily taken up by the lutein cells and stored by them for long periods, so that the corpora lutea take on an intense blue color; that the dye is not unduly toxic in proper doses, and that with certain precautions it withstands the manipulations necessary to the preparation of paraffin sections. Furthermore, a long series of experiments by Evans and Long proves that the dye as used for vital staining of the ovaries does not of itself inhibit ovulation in rats.

The animals were from a stock resulting from the inter-breeding of albinos with a few rats of the ordinary brown variety. On the day of littering and three succeeding days they were given intra-abdominal injections of 4 cc. of a sterilized 1 per cent solution of the dye in 0.7 per cent NaCl. On the fifth day we began the administration of corpus luteum substance. Two preparations were used, one being Armour's desiccated corpus luteum substance from cows, extracted with petroleum benzene to remove the fats (the method of preparation is given by Fenger (6)); the other was that prepared by Hynson, Westcott & Dunning, which we are informed by the makers undergoes no changes in its preparation except such as are incidental to the careful desiccation. We are very much indebted to the firms mentioned for their courtesy in furnishing the materials for our work. In administering the corpus luteum substance all aseptic precautions were used except that the powder itself could not be sterilized. The powder was suspended in sterile normal saline solution by means of a mortar and pestle.

Ten rats, stained as described, were each given ten doses of the Armour preparation on alternative days, each dose consisting of 200 mgm. of corpus luteum substance in 3 to 4 cc. of normal saline. As the average body weight of the animals was 175 grams, the dose represented somewhat more than 1 gram per kilogram of body weight, and the total amount received by each rat was 4 grams, equivalent to about 16 grams of the fresh substance.

On the twenty-fifth day after littering the animals were killed, there having been time for two, three or four ovulation cycles to have elapsed, according to individual variations of the rats. All had gained a little in weight (they were young animals) and autopsy showed all but one to be in good condition, except for intra-abdominal adhesions due to the granular, partly insoluble and unsterile nature of the injected substance. The material had been well absorbed in all cases. One rat showed a severe recent peritonitis. The numbers of stained and unstained corpora lutea were noted and then carefully checked under the microscope in serial sections; to which end the ovaries were fixed by injection of Zenker's fluid through the aorta, removed after three hours and washed in 70 per cent alcohol, dehydrated, cleared with xylol and imbedded in paraffin. The serial sections were stained on the slide with alcoholic carmine. It is important to avoid aqueous reagents in order not to dissolve the microscopic dye-granules.

In all the animals, including the one which was suffering from peritonitis, ovulation had continued unchecked, as proven by the presence in their ovaries of new unstained corpora lutea numbering from thirteen to thirty. The individual counts of stained and unstained corpora are very similar to those found in stained animals which have not received corpus luteum substance, after the same number of days. In one of the experimental animals the normal ova of a recent ovulation were discovered in the Fallopian tubes.

One rat received the Hynson, Westcott & Dunning preparation, but in daily doses, thus receiving twice as much as was given the first ten animals. This dose was sufficient to cause great emaciation and many adhesions, but there were ten unstained and thirteen stained corpora lutea in the two ovaries. A control animal killed the same number of days after littering showed thirteen unstained and twenty-seven stained corpora lutea.

CONCLUSION

The intraperitoneal injection of large doses of desiccated mammalian corpus luteum substance does not inhibit ovulation in the rat.

BIBLIOGRAPHY

(1) BEARD: Anat. Anz., 1898, xiv, 97.
(2) PRENANT: Rev. Gen. d. Sci., 1898, ix, 646.
(3 LOEB: Deutsch. med. Wochenschr., 1911, i.
(4) STOCKARD AND PAPANICOLAOU: Amer. Journ. Anat., 1917, xxii, 225.
(5) PEARL AND SURFACE: Journ. Biol. Chem., 1914, xix, 263.
(6) FENGER: Journ. Amer. Med. Assoc., 1914, lxii, 1249.

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